Sitka Tribe of Alaska
Quality Assurance Plan
for the
Traditional Foods Heavy Metal Baseline Study

Prepared by
Sitka Tribe of Alaska
Resource Protection Department
for the
USEPA's Indian General Assistance Program
August 2013

Approval:

_____ _____
Chris Whitehead, Technical Lead, Sitka Tribe Date

_____ _____
Jeff Feldpausch, Program Director, Sitka Tribe Date

_____ _____
Michelle Davis, USEPA Region 10 Grant Coordinator Date

_____ _____
Robert Gerlach, Environmental Health Lab, AK Date

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Distribution List and Contact Information

Name	Organization	Address	Phone No	Fax No	E-mail Address
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Jeff Feldpausch	Sitka Tribe of Alaska	456 Katlian Street, Sitka AK 99835	907-747-7469	907-747-7506	jeff.feldpausch@sitkatribe-nsn.gov
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Robert Gerlach	AK Health Lab	5251 DR MLK Jr. Ave Anchorage, AK 99507	907-375-8214		bob.gerlach@alaska.gov

1 Project Management and Organization

This project is a multi-agency collaboration designed to determine baseline heavy metal data within traditional marine foods for the Sitka Tribe of Alaska. . The organizations involved in this project are:

Sitka Tribe of Alaska (STA): Chris Whitehead shall be responsible for submission of the QA to EPA for review and approval, and maintenance and preparation of program reports and deliverables to EPA. He will also be the main contact for EPA requests. Mr. Whitehead is also responsible for the sampling design and oversight of the sample collection and analyses, data analysis and database input. He will also be responsible for the coordination of field and laboratory analyses of samples.

US Environmental Protection Agency: The US EPA Project Coordinator shall be Mrs. Michelle Davis. Mrs. Davis shall be responsible for the oversight of the project and shall ensure that the goals and objectives of the projects are achieved. She shall review and approve the QA and subsequent addendums or amendments submitted to EPA. She shall ensure that project deliverables are complete and of necessary quality and that the project completion dates are met.

1.1 Roles and Responsibilities

Sitka Tribe of Alaska:
Points of Contact: Jeff Feldpausch, Chris Whitehead. STA shall be responsible for the following tasks:

- finfish and shellfish sample collection
- processing and shipment of samples to laboratories
- quantify levels of specific heavy metals in each sample
- coordination of sample analyses
- data validation
- data input to database
- data analysis/interpretation
- report writing

Alaska State Environmental Health Laboratory: Point of Contact: Robert Gerlach. Mr. Gerlach shall be responsible heavy metal level testing of the collected tissue samples.

1.2 Project Definition

The proposed project will focus on sampling shellfish and finfish for the determination of toxins related to heavy metals. The main goal of this project is to provide baseline data that can be used in future studies regarding traditional subsistence foods.

1.2.1 Heavy Metal toxins

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis (Roberts, 1999).

This baseline data collection project will evaluate the following metals in specific traditional foods (table. 1):

Table 1. Heavy metals by species

	Heavy Metal						
Species	Arsenic As	Cadmium Cd	Chromium Cr	Copper Cu	Nickel Ni	Lead Pb	Selenium Se
Chinook Salmon							
Coho Salmon							
Sockeye Salmon							
Pink Salmon							
Chum Salmon							
Pacific Herring							
Lingcod							
Pacific Halibut							
Yelloweye Rockfish							
Dungeness crab							
Littleneck clam							
Butter clam							
Geoduck clam							
Chiton							
Seal							

1.2.2 Measurable Project Objectives

The measurable objectives for this project are as follows:

1. Collect baseline data on heavy metal levels in specific traditional subsistence species.
2. Input data in to database to be used in future studies.

1.3 Schedule of Project Task/Activities

Project Task	Estimated Start Date	Estimated Completion Date	Comments
QA Submission to EPA	August, 2013	September,2013	
QA Review and Approval	September,2013	September,2013	
Tissue Sample Collection	September,2013	August, 2014	Bi-monthly
Tissue Sample Analysis	September,2013	August, 2014	Bi-monthly
Data Input	September,2013	August, 2014	Bi-monthly
Data Analysis	December,2013	September,2014	Quarterly
Report Writing	December,2013	September,2014	Quarterly

1.4 Documentation and Records

Complete documentation for inspections may include but are not limited to the following forms to be completed and collated by STA:

- Filed Sampling Report
- Record of Sampling
- Laboratory Analysis Reports

The field team will maintain field notes in a bound notebook and all documents, records, and data collected will be kept in a case file and submitted to the program office with the final project report.

The following documents will be archived at the Sitka Tribe of Alaska Resource Protection Department office and the designated laboratories performing the analyses: (1) hard copies of sampling sheet (2) electronic and hard copy of analytical data.

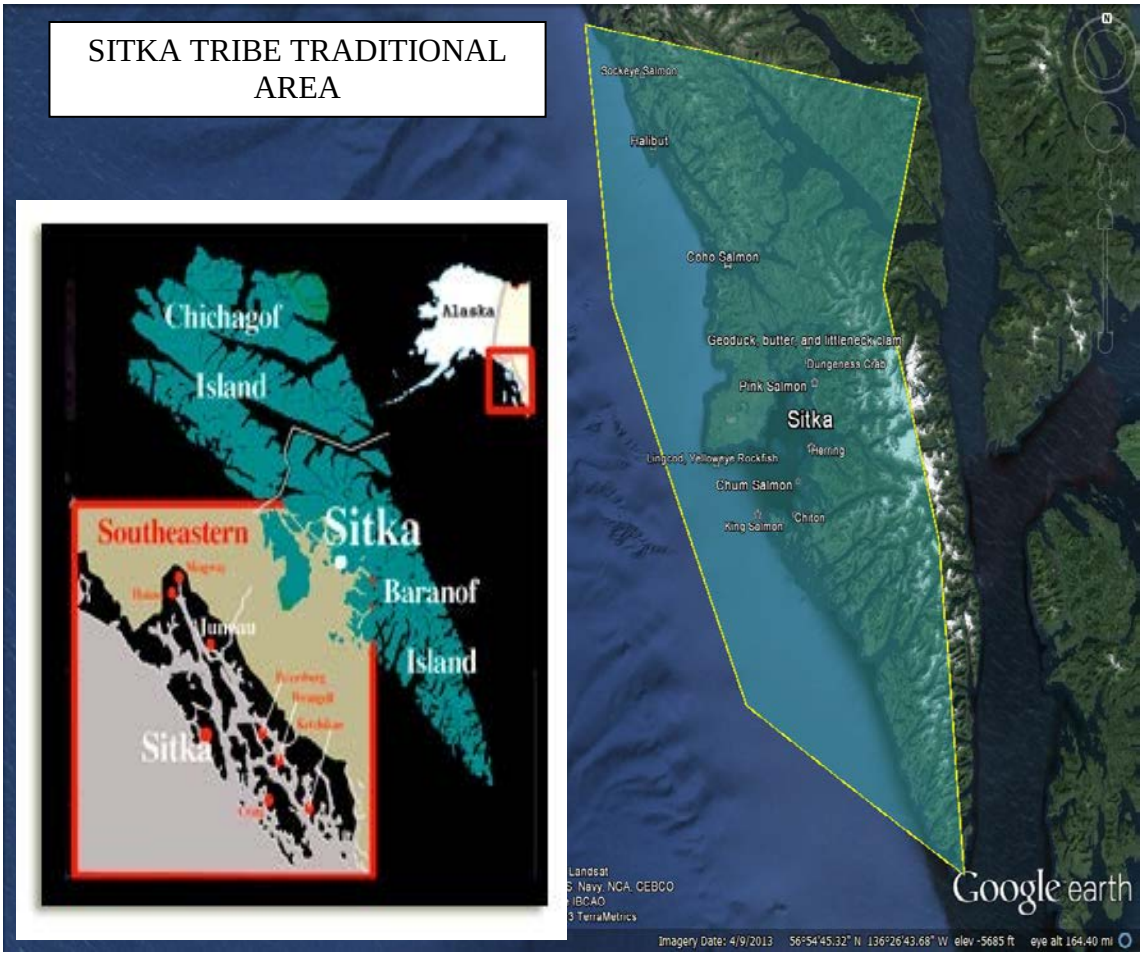
The laboratory shall store all sample receipt, sample login, extraction/preparation, and laboratory instrument print-outs and other analytical documentation as per their established SOP.

2 Measurement and Data Acquisition

2.1 Sample Locations

Shellfish and finfish tissue samples will be collected at traditional harvest sites within the traditional area of the Sitka Tribe (Figure 1):

Figure 1: Sample Collection Locations



2.2 Sample Collection

A minimum of two STA staff members will use a small boat to access collection sites within the traditional area of the Sitka Tribe of Alaska (see Figure 1 above). Each sampling site location will be recorded using a global positioning system (GPS). Environmental factors such as weather, date and time, tide, and sample depth will be recorded. Samples will be collected twice per month (minimum). Most species are seasonal and sampling times will vary (see Table 2).

Table 2. Sample Season

Species	Sample Season Start	Sample Season End
Chinook Salmon	June	September
Coho Salmon	August	October
Sockeye Salmon	June	September
Pink Salmon	July	September
Chum Salmon	July	September
Pacific Herring	March	April
Lingcod	May	October
Pacific Halibut	May	October
Yelloweye Rockfish	May	October
Dungeness crab	June	November
Littleneck clam	Year round during low tide series	
Butter clam	Year round during low tide series	
Geoduck clam	Year round during low tide series	
Chiton	Year round during low tide series	
Seal	May	January

Samples will be shipped with a chain of custody (COC) generated by the project technical lead. The COC will specify sample numbers, date and time of collection, sample matrix, parameters for analysis, and any pertinent observations or comments.

All shellfish and finfish samples collected during this study will be analyzed for heavy metal toxins using Alaska Department of Health Environmental Lab sampling and analysis methods. Finfish samples will be skinless and taken from behind the head. Sufficient tissue samples will be collected to yield an approximate amount of at least ½ pound of fish meat for processing.

Shellfish sample sizes will vary and may range from 15-20 clams per sampling site depending on species.Specimens must not be cracked or have crushed shells. Samples will not be held in freshwater or seawater at anytime after collection.

Samples will be frozen prior to shipment. A standard data collection form containing species, date, harvest site, length and weight will be sent along with the sample. Samples will be placed in a labeled bag with date and location of collection and shipped in a Styrofoam cooler inside a cardboard box. Ice packs will be used to keep the sample cool.

Samples will be sent to the Environmental Health Laboratory at 1521 Dr. MLK Jr. Ave, Anchorage, AK 99507.

2.3 Decontamination Procedures

Samples will be collected using clean sampling devices and sample collection gears. As much as possible, disposable sampling equipment and gear shall be used. Sampling devices and sample collection gear like rain gear, and rubber boots will be cleaned and decontaminated using disinfectants. Samplers will follow the proper health and safety procedures when collecting and handling samples to minimize or not to incur contamination.

2.4 Sample Handling and Shipping

Sample custody and documentation will be consistent with established EPA protocols. Samples will be labeled with information including the sample number, date, and time.

Information about each sample will be entered on a chain of custody form that will accompany the samples to the laboratory.

Packaging, marking, labeling, and shipping of samples will comply with all regulations promulgated by the U. S. Department of Transportation (DOT) in the Code of Federal Regulations, 49 CFR 171 – 177 and International Air Transport Association (IATA) regulations.

2.5 Analytical Methods

Summary of Laboratory Measurements, Methods, Target Detection Limits and Expected Ranges for heavy metals in samples are listed in Environmental Health Lab Standard Operating Procedures (Appendix 1.).

2.5.1 Quality Control

Variation for laboratory analysis will be assessed through standard laboratory protocols (see individual laboratory SOPs).

3 Assessment and Response

Alaska Environmental Health Lab is a certified laboratory, no other certifications are needed.

Deviations from the QA plan shall be documented in a Sample Alteration Form (Attachment 1). Problems encountered in the field or laboratories shall be resolved and documented in a Corrective Action Form (Attachment 2). Both Sample Alteration and Corrective Action Forms shall be reviewed and approved by USEPA prior to implementation. Reports and other required documentation shall be furnished to EPA at the required frequency and schedule.

4 Data Validation and Interpretation

Standard laboratory procedures for analytical data reduction, review and reporting will be followed. The Environmental Health Lab will immediately inform the project technical lead of any problems with sample shipment conditions, holding times, or analyses. Analytical data shall be peer reviewed by the laboratories prior to submission to STA.

Data will be sent from laboratory to the project technical lead electronically or on paper lab slips. Lab and field analytical data will be matched with sample times and locations. All data will be screened for questionable values and problems and inspected for missing or improbable data or results.

All data collected during the project will be entered into an Excel database at the Sitka Tribe Resource Protection office after the data have been reviewed for quality assurance. Results will be graphed to portray seasonal variations by species as well as harvest site comparisons. Regression analyses and other appropriate statistical tests will be conducted to investigate possible correlations between all source data types. All analyses will be provided in a final report to the EPA Project Officer. Raw data will also be available in the final report or upon request.

5 References

Roberts JR. June 1999 Training Manual on Pediatric Environmental Health: Putting It into Practice. (http://www.cehn.org/cehn/trainingmanual/pdf/manual-full.pdf) Children's Environmental Health Network, Emeryville, CA, U.S.A.

Attachment 1 – Sample Alteration Form

Project Name and Number: _____

Sample Matrix: _____

Measurement Parameter: _____

Standard Procedure for Field Collection & Laboratory Analysis (cite reference):

_____

_____

Reason for Change in Field Procedure or Analysis Variation:

_____

_____

Variation from Field or Analytical Procedure:

_____

_____

Special Equipment, Materials or Personnel Required:

_____

_____

Initiators Name:_____ Date: _____

Project Officer:_____ Date: _____

QA Staff:_____ Date: _____

Attachment 2 – Corrective Action Form

Project Name and Number:_____

Sample Dates Involved:_____

Measurement Parameter:_____

Acceptable Data Range:_____

Problem Areas Requiring Corrective Action:__ _____

Measures Required to Correct Problem(s):

_____

Means of Detecting Problems and Verifying Correction:

_____

Initiators Name:_____ Date:_____

Project Officer:_____ Date: _____

Quality Staff:_____ Date: _____

Appendix 1

Standard Operating Procedures

ALASKA
Department of
Environmental
Conservation

STANDARD OPERATING PROCEDURE
ACID DIGESTION OF SEDIMENTS, SLUDGES, FISH TISSUE, AND SOILS BY EPA 3050B

Prepared by: _____ Date: _____
Xuedong Man and Vivianne Sawasaki
Chemist III Technician

Reviewed by: _____ Date: _____
Emanuel Hignutt, Jr.
Chemist IV

Reviewed by: _____ Date: _____
Shera Hickman
Quality Systems Manager (Acting)

Approved by: _____ Date: _____
Thomas K. Hathaway, Ph.D.
Chief of Laboratory Services

STATE OF ALASKA
DEPARTMENT OF ENVIRONMENTAL CONSERVATION
ENVIRONMENTAL HEALTH LABORATORY
5251 HINKLE ROAD, ANCHORAGE, AK 99507

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5.1.1 **Page**

1.0 Scope and Application

1.1 This SOP has been written to provide the digestion procedures for the preparation of sediments, sludges, fish tissue and soil samples for analysis of samples by inductively coupled plasma mass spectrometry (PerkinElmer Elan ICP-MS DRC II).This method is not a total digestion technique for most samples. It is a very strong acid digestion that will dissolve almost all elements that could become "environmentally available." By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment. For the digestion of samples, a representative 0.5-1 gram (wet weight) or 0.5 gram (dry weight) sample is digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide. The resultant digestate is reduced in volume while heating and then diluted to a final volume of 50mL.

2.0 Summary of Method

2.1 A 0.5g to 1.0 gram portion of sample (fish tissue or soil) is digested with concentrated HNO_3 and refluxed until no brown fumes are given off indicating the complete reaction with HNO_3. Water and 30% H_2O_2 are then added and the refluxing is continued until the effervescence subsides. Heating then continues for an additional 2 hours after which the sample is brought up to a final volume with water and the sample analyzed for trace metals individually detected and quantified by ICP-MS.

3.0 Health and Safety Warnings

3.1 Before performing this SOP, the pertinent MSDS for each chemical should be read.

3.2 Gloves, eye protection glasses and lab coat should be worn at all times while performing sample extraction steps, or when handling samples or extracts

3.3 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. All expired stock and working standards must be disposed of by current ADEC EHL protocol.

3.4 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

3.5 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

3.6 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection for UV emissions.

4.0 Cautions

4.1 During digestion, do not allow the sample to boil or evaporate to dryness. Keep sample covered with a watch glass while refluxing to avoid analyte loss.

5.0 Interferences

5.1 Sludge and fish tissue samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed in accordance with the quality control requirements given in this SOP to aid in determining whether Method 3050B is applicable to a given sample.

6.0 Personnel Qualifications

6.1 This procedure may only be performed by an EHL Laboratory Technician or Chemist I-IV. Analysts independently performing this method must complete an Initial Demonstration of Capability. Analysts who have not completed an Initial Demonstration of Capability may perform the method only under the direct supervision of the Chemistry Supervisor or other qualified staff.

7.0 Sample Collection, Handling and Preservation

7.1 Fish Tissue: For the determination of elements in fish tissues, biologists from International Pacific Halibut Commission (IPHC), Alaska Department of Fish and Game (ADF&G), Nation Oceanic and Atmospheric Administration (NOAA), and commercial and native fisher-men or others will collect samples from predetermined locations. Skinless fillets are homogenized. The homogenized tissue is stored in wide mouth jars in the freezer at ≤ -10°C until the day before analysis. Then it is thawed in the refrigerator until it is weighed out. The unused portion is returned to the freezer.

7.2 Water samples: For the determination of total recoverable elements in aqueous samples, samples are not filtered. The samples are acidified with nitric acid to pH <2 (or are received already acidified). The typical sample size is 1L. The samples are stored at room temperature.

7.3 Soil and other solid samples: For the determination of total recoverable elements in solid samples, samples are stored at 4ºC. The typical sample size is 50g, but may be smaller.

7.4 Extracts are stored at 4°C for at least 30 days, or until such time as it is determined that no further analysis be needed. Once confirmed that no more analysis will be needed the samples may be disposed.

8.0 Equipment and Supplies

8.1 PerkinElmer Elan ICP-MS DRC II.

8.2 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

8.3 Analytical balance, with capability to measure to 0.0001 g, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

8.4 A temperature adjustable hotblock capable of maintaining a temperature of 95°C.

8.5 A gravity convection drying oven with thermostatic control capable of maintaining 105°C ± 5°C

8.6 Air displacement pipetters capable of delivering an assortment of volumes ranging from 0.1-2500 µL

8.7 An assortment of high quality disposable pipet tips appropriate for the pipetters used.

8.8 Labware:

8.8.1 Glassware – volumetric flasks.

8.8.2 Environmental Express 68mL or 125mL digestion cups with screw caps and watch glasses.

8.8.3 50mL and 15mL conical tubes.

8.8.4 Narrow-mouth storage bottles, plastic with screw closure.

8.8.5 One-piece stem plastic wash bottle with screw closure, 125 mL.

8.8.6 Squeeze bottle for DI (reagent) water.

9.0 Reagents and Standards

9.1 Reagent water - All references to reagent grade water in this SOP refer to ASTM Type I water (ASTM D1193).

9.2 Reagent Acids:

9.2.1 Nitric acid, Ultrex II Ultrapure Reagent HNO_3, Fisher Scientific.

9.2.2 Hydrochloric acid, Ultrex II Ultrapure Reagent HCl, Fisher Scientific.

9.2.3 Hydrogen peroxide (30%), Ultrex II Ultrapure Reagent H_2O_2, Fisher Scientific.

9.3 1% Nitric Acid (HNO_3) solution: add 20 mL concentrated HNO_3 solution to 2L DI (reagent) water. This is used for making the ICV, CCV, LCS, and calibration standards.

9.4 Standard Stock Solutions - Stock standards may be purchased from a reputable commercial source. Stock solutions should be stored in plastic (such as polyethylene) bottles. Replace stock standards before the expiration date.

9.5 Calibration Standards:

9.5.1 Intermediate Calibration Standard: Dilute the 100 mg/L stock standard AccuStandard (ICP-MS-200.8-Cal 1R) to make the calibration standards at 1, 10, 100, and 300µg/L.

9.6 Tuning Solution (6020TS –Inorganic Ventures): 1ppb Elan 6100DRC setup solution.

9.7 Daily Performance Check Solution (made by diluting Inorganic Ventures standards of Mg, In, U, Ce, and Ba): 1ppb Elan 6100 DRC sensitivity detection limit solution.

10.0 Sample Preparation

10.1 Mix the sample thoroughly to achieve homogeneity. All equipment used for homogenization should be cleaned with 1% HNO_3 to minimize the potential of cross-contamination.

10.2 For each digestion procedure, weigh to the nearest 0.01 g and transfer a 0.5-1.0 g sample (wet weight) or 0.5 g sample (dry weight) to a 68mL digestion vessel. For samples with high liquid content, a larger sample size may be used provided digestion is complete.

10.3. Add 5 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to 95ºC ± 5ºC and reflux for 10 to 15 minutes without boiling.

10.4 Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL of conc. HNO_3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO_3.

10.5 Cover the vessel with a ribbed watch glass heat at 95ºC ± 5ºC without boiling for 1.5 hours. Maintain a covering of solution over the bottom of the vessel at all time.

10.6 Add 2 mL of water and 0.5 mL of 30% H_2O_2. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat the solution until the effervescence subsides, allow the vessel to cool.

10.7 Continue to add 30% H_2O_2 in 0.5-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. Continue 30% H_2O_2 additions for no more than 30 minutes.

10.8 Cover the sample with a ribbed watch glass or vapor recovery device heat the acid-peroxide digestate at 95ºC ± 5ºC without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

10.9 After cooling, bring sample to 20 mL with DI H_2O, stir, and draw out 10mL and place into a new 68mL digestion vessel. Bring the volume of that portion up to 25mL with DI H_2O – this portion will be used to analyze As, Se, Cd, and Pb. To the remaining portion of add 2.5mL of HCl and reflux for 15 minutes. Allow sample to cool and bring the volume to 25mL with DI H_2O – this portion will be used to analyze Cr and Ni. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by ICP-MS.

10.10 Alternatively, weigh out 1.0-2.0g of sample (wet weight) or 1.0g (dry weight) into a 125mL digestion vessel and add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to 95ºC ± 5ºC and reflux for 10 to 15 minutes without boiling.

10.11 Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL of conc. HNO_3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO_3.

10.12 Cover the vessel with a ribbed watch glass heat at 95ºC ± 5ºC without boiling for 2 hours. Maintain a covering of solution over the bottom of the vessel at all times.

10.13 Add 2 mL of water and 1 mL of 30% H_2O_2. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat the solution until the effervescence subsides, allow the vessel to cool.

10.14 Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. Do not add more than a total of 10 mL 30% H_2O_2.

10.15 Cover the sample with a ribbed watch glass or vapor recovery device heat the acid-peroxide digestate at 95ºC ± 5ºC without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

10.16 After cooling, dilute to 100 mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by ICP-MS.

11.0 Data Analysis and Calculations

11.1 Calculations:

Sample Concentration

$$\text{Concentration } (\mu g/g) = \frac{(Df)(\text{ppm found})}{}$$

Where:
Df = Dilution factor. If no dilution was made, D = 1.

Percent Drift (%D)

$$\%\text{Drift} = \frac{|C_c - C_t|}{(C_t)} * 100\%$$

Where:
C_c= Calculated concentration
C_t= Theoretical concentration

LCS Percent Recovery

$$\%R = \frac{(C_s)}{(C_a)} * 100$$

Where:
C_s = Observed spike concentration
C_a = Spike Level

Duplicate Relative Percent Difference (RPD)

$$RPD = \frac{|X_1 - X_2| * 100}{(X_1 + X_2)/2}$$

Where:
X_1 = Concentration of sample
X_2 = Concentration of duplicate

12.0 Data and Records Management

12.1 Data Handling: All raw batch data must be recorded in Metals Prep Logbook. The sheet containing the sample preparation work must be reviewed, signed and dated by the analyst. All the raw results will undergo secondary review by Section Supervisor (primary), QA Manager, or Chief before being released by the Environmental Health Laboratory.

13.0 Quality Control

13.1 IDC/MDL Studies: An IDC must be conducted by each new analyst. An MDL study must be conducted annually. If appropriate, the IDC and MDL studies may be combined.

13.1.1 Four passing LCS's must be provided to validate an analyst for this method.

13.1.2 An MDL study should be conducted when significant changes in instrument occur, or when a new instrument is purchased for the analysis.

13.2 EPA 6020A specific Criteria: All masses which might affect data quality must be monitored. Isobaric polyatomic ion interference must be recognized and appropriate corrections made to the data. Dilute and rerun samples that are more concentrated than the linear or measure an alternate less-abundant isotope.

13.2.1 Interference Check Solution should be run every 12 hours during active analysis of samples.

13.2.2 One Dilution test should be run every 20 samples. The result must be within 10% of the original measurement.

13.2.3 A post-digestion spike should be run if matrix interference is suspected in a sample. The result should be within 15% of the original measurement plus the spiked amount.

13.3 Initial Calibration Verification:

13.3.1 The ICV acceptance criterion is +/-10%.

13.3.2 The concentration of standards should range from 10µg/L to 200µg/L.

13.4 Continuing Calibration Verification (CCV): The 100µg/L is used as working CCV and analyzed at the beginning of a run, after every 10 samples and at the end of the run.

13.4.1 The CCV acceptance criterion is +/-10%. If the recovery is not within +/-10%, recalibrate and rerun all the samples since last compliant continuing calibration standard.

13.5 Method Blank/Calibration Blank (MB/CB): A calibration blank must be analyzed after the CCV. The first CB of a run is labeled as MB.

13.5.1 The acceptance criterion is ≤ the ½RL.

13.5.2 When MB/CB contamination is apparent, samples associated with the MB/CB may require reanalysis.

13.5.3 If the measured sample concentration is >10X the MB/CB contamination, the sample does not need reanalysis. If the sample has a concentration < RL, the sample does not need reanalysis. All other associated samples will require reanalysis.

13.6 Laboratory Fortified Blank or Laboratory Control Standard (LFB or LCS): The 100µg/L standard is used as LCS and analyzed with each batch of 20 samples.

13.6.1 An LCS spike standard is made by diluting 1mL each of 1000µg/mL Inorganic Ventures stock standard Arsenic, Cadmium, Chromium, Lead, Nickel, and 5mL of Selenium.

13.6.2 The advisory acceptance criterion is +/- 20 % recovery of the true value. Alternatively control chart limits will be established for each element.

13.6.3 If the recovery is outside of the acceptance goal, one rerun is allowed. If the recovery remains unacceptable, re-digestion of the batch may be necessary or the data will be qualified with a comment regarding LCS recoveries outside of acceptance criteria.

13.7 Sample Duplicate: A sample duplicate is performed every ten samples or only at the request of the client.

13.7.1 The Relative Percent Difference (RPD) for sample/duplicate is ≤ 20%.

13.7.2 A reanalysis is required when the allowable RPD limit is exceeded. If the rerun batch has a RPD > 20%, the sample will be flagged as non-homogeneous.

13.8 Matrix Spike and Matrix Spike Duplicate: A pair of matrix spike and matrix spike duplicate are analyzed with every 20 samples.

13.8.1 The MS/MSD spiking standard is freshly prepared every day and is the same source standard as the LCS with a concentration between low and midlevel calibration standards.

13.8.2 The MS recovery limit is 70-130%.

13.8.3 If matrix spike recovery falls outside the method established limits of 70-130% and lab performance is otherwise in control, then the data may be labeled suspect due to matrix effects.

14.0 References

14.1 USEPA, Methods 3050B, Revision 2, December 1996.

14.2 USEPA, Methods 6020A, Revision 1, January 1998.

STANDARD OPERATING PROCEDURE

	Signature and Title	**Date**
Prepared by:	Christopher Rivera Laboratory Technician	
Reviewed by:	Vivianne Sawasaki, Microbiologist I	
Reviewed by:	Emanuel Hignutt, Jr., Chemist IV	
Approved by:	Patryce McKinney Chief of Laboratory Services	

Periodic Review:

Signature	**Title**	**Date**

_____	_____	_____
_____	_____	_____
_____	_____	_____

This is a controlled document with the most recent updated revision. This SOP should be reviewed on an annual basis. If the SOP is found adequate, the SOP cover page is signed and dated for documenting the review. If major revisions are needed, a new revision will be released with a new signature page. The above signatures reflect periodic review of the Standard Operating Procedure.

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Tables

2.0 Scope and Application

1.1 This method is applicable to the determination of total mercury in various environmental matrices including biota, sediments, and soil samples.

3.0 Summary of Method

3.3 Controlled heating in an oxygenated decomposition furnace is used to liberate mercury from solid and aqueous samples in the instrument. The sample is dried and then thermally and chemically decomposed within the decomposition furnace. The decomposition products are carried by flowing oxygen to the catalytic section of the furnace. Here oxidation is completed and halogens and nitrogen/sulfur oxides are trapped. The remaining decomposition products are then carried to an amalgamator that selectively traps mercury. After the system is flushed with oxygen to remove any remaining gases or decomposition products, the amalgamator is rapidly heated, releasing mercury vapor. Flowing oxygen carries the mercury vapor through absorbance cells positioned in the light path of a single wavelength atomic absorption spectrophotometer. Absorbance (peak height or peak area) is measured at 253.7nm as a function of mercury concentration.

4.0 Health and Safety Warnings

4.3 Before performing this SOP, the pertinent MSDS for each chemical should be read.

4.3 Proper personal protective equipment (PPE) should be worn at all times while handling reagents, standards, and samples.

4.3 Use care in handling hot quartz boats that have recently gone through the analysis or cleaning procedures.

4.3 Many mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Extreme care must be exercised in the handling of concentrated mercury reagents. Concentrated mercury reagents should only be handled by analysts knowledgeable of their risks and of safe handling procedure. All expired Hg stock and working standards must be disposed of per the EHL Chemical Hygiene Plan.

4.3 Use acceptable procedures for handling the compressed oxygen gas. When moving and replacing the oxygen bottle, ensure that the valve has been covered with the protective cap. Never "blow out" the valve of a new tank when replacing the regulator. Never use Teflon™ tape. The tank valve should only be on when the instrument is on. After installing a new oxygen bottle, check for leaks. Always transport new and used tanks with an approved bottle cart.

4.3 Even though the amount of mercury exiting the instrument is small, a mercury trap should be employed in a vented fume hood

5.0 Interferences

5.3 Carryover (memory effects) between analyses may be encountered when analyzing a sample of high mercury concentration (400ng) prior to analyzing one of low concentration (25ng or less). Typically, to minimize memory effects, analyze the samples in batches of low and high concentrations, always analyzing those of low concentration first. If this batching process cannot be accomplished, a blank analysis with an extended decomposition time may be required following the analysis of a highly concentrated sample to limit memory effects, or several empty boats or empty slots are analyzed until the result is below the MDL.

6.0 Personnel Qualifications

6.3 This procedure may only be performed by an analyst trained in using the DMA-80 and has run four passing IDC's. Analysts who have not completed an Initial Demonstration of Capability may perform the method only under the direct supervision of the Chemistry Supervisor or other qualified staff.

7.0 Sample Collection, Handling and Preservation

7.3 Solid samples should be submitted in pre-cleaned glass jars (I-Chem, Eagle Picher, or equivalent) and are stored at 4°C until analysis.

7.3 Biota samples are stored in wide mouth jars in the freezer at ≤ -10°C until the day before analysis.

7.3 Fish tissue samples are thawed overnight at 4°C and homogenized with a small, stainless spatula by stirring; then loaded onto quartz sample boats of the DMA-80 automatic mercury analyzer.

8.0 Equipment and Supplies

8.3 Milestone Model DMA-80 Direct Mercury Analyzer, or equivalent.

8.3 Analytical balance, capable of weighing to 0.1mg.

8.3 Milestone quartz sample boats, or equivalent.

8.3 A muffle furnace capable of maintaining a temperature of 800˚ C.

8.3 Stainless steel tongs.

8.3 A gravity convection drying oven with thermostatic control capable of maintaining 110˚C.

8.3 Desiccator.

8.3 Glassware – volumetric flasks, class A, 10mL.

8.3 Porcelain crucibles for quartz boat storage.

8.3 Assorted calibrated pipettes.

8.3 Teflon coated weighing spatula.

8.3 Stainless stirring spatula.

8.3 Small cleaning brush.

8.3 Squeeze bottle with DI water.

9.0 Reagents and Standards

9.3 Reagent Water, ASTM Type II, Milli-Q or equivalent.

9.3 1% Nitric Acid (HNO_3)

Add 20mL concentrated HNO_3 solution to approximately 1500mL Milli-Q (reagent) water. Mix well and dilute to 2000mL with Milli-Q (reagent) water.

9.3 Stock Mercury (Hg) Standard, 1000mg/L Hg (Inorganic Ventures, catalog #CGHG1-1 or equivalent).

9.2.1 10mg/L Mercury (Hg) Intermediate Calibration Standard: Dilute 0.1mL (100µL) of 1000 mg/L Stock Mercury (Hg) Standard to 10mL with 1% nitric acid.

9.2.2 1.0mg/L Mercury (Hg) Working Calibration Standard: Dilute 1mL of the 10mg/L Mercury (Hg) Intermediate Calibration Standard to 10mL with 1% nitric acid.

9.2.3 0.1mg/L Mercury (Hg) Working Calibration Standard: Dilute 1mL of the 1.0mg/L Mercury (Hg) Intermediate Calibration Standard to 10mL with 1% nitric acid.

9.3 Certified Reference Material: Dogfish Muscle Certified Reference Material for Trace Metals, DORM-3, National Research Council Canada. This CRM is used for the Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) (NRC-CNRC, catalog #DORM-3 or equivalent).

9.3 Oxygen, Ultra High Purity (UHP) grade.

10.0 Instrument Setup, Calibration and Standardization

10.3 Set instrument parameters according to the conditions listed in Table 1.

10.3 Calibration.

10.2.1 Login as the administrator to perform calibrations.

10.2.2 On the main DMA-80 desktop page, select the DMA-80 Measurement. Click the **Calib.** tab and press the "diskette" icon to save the current calibration to a new data file (Example: "010511a"). Clear the calibration points by pressing the "blank paper" icon and resave the data file.

10.2.3 Return to the **Meas.** tab and click the "magic wand" icon to add a new line. In the Sample Name field type the ICAL standard name. Under the Weight column, enter the volume in mL of the working Hg standard that will be used for

calibration (see Table 4). Under the State drop-down menu select calibration. "C" will appear in the State column, indicating that the current process is calibration.

10.2.4 Select the **Links** tab and choose the correct calibration and method files.

10.2.5 Place the sample quartz boat into the position indicated in the "Pos" column, and then pipette into the sample boat the proper volume of Hg calibration standard solution as in Table 4. Each standard solution is analyzed twice.

10.2.6 Select the **Result** tab, and press the **Start** button to begin calibration. Once finished running all the calibration points, press the "diskette" icon to confirm the name of the data file and save. Select the **Calibration** tab. Press the **Cell 1** tab, select "linear calibration algorithm with $R^2 \geq 0.990$." Repeat the same procedure for **Cell 2**. Press the "diskette" icon to confirm the name of the data file and save.

10.2.7 If there is difficulty meeting the linear or quadratic calibration algorithm with $R^2 \geq 0.990$, the instrument will require recalibration and/or replacement of the catalyst/amalgamator system and maintenance. Consult the DMA-80 operating manual for the details of the other two calibration approaches.

11.0 Sample Preparation

11.3 Samples: Transfer approximately 0.2g ± 0.02g of sample into a quartz boat.

11.3 Method Blank: Place an empty quartz boat in the DMA-80.

11.3 Calibration Blank: Pipette 20µL of 1% nitric acid into a quartz boat. **Calibration only!**

11.3 Continuing Calibration Verification: The CCV's are analyzed in tandem using the DORM-3 CRM: Transfer 0.04g ± 0.004g of Dorm-3 CRM into a sample quartz boat for the low level cell CCV and 0.2g ± 0.02g for the high level cell CCV.

11.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD) set: Weigh out two separate 0.2g ± 0.02g aliquots of a sample from the batch for the MS/MSD set. Spike the MS/MSD samples with 50µL of 1.0mg/L Mercury (Hg) Working Calibration Standard.

12.0 Sample Analysis

12.3 Power up the DMA-80 Mercury Analyzer (switch at right front of instrument).

12.3 Turn on the oxygen supply at the tank and adjust the low pressure side of the regulator to 60 psi and 600 psi to the high pressure side (the DMA-80 must be on prior to this step).

12.3 Perform a calibration check of the balance in accordance with the appropriate SOP (5, 1, 0.2, and 0.005 grams).

12.3 After letting the DMA-80 warm up for at least 30 minutes, log on under your name with the appropriate password. Select DMA-80 Measurement and click on the **Meth.** tab to

select the appropriate method. Verify that the instrument settings are correct (see Table 1 and Table 2). Click on the **System** tab to verify all levels are red.

12.2.1 Be sure the Transcend memory card is inserted in the instrument prior to running a batch on the DMA.

12.3 Under the **Meas.** tab select the **Sample** tab and click the 'blank page" icon. Click the "diskette" icon. Save the data file as the date of analysis followed by an "a" (Example: "020310a"). Additional runs can be saved as the date of analysis and the next letter in the alphabet. This is to identify different runs if there are multiple runs in a single day (Example: "100810a, 100810b, 100810c,"etc).

12.3 Initially, there must be two or three blank analyses (with or without a boat) to ensure that there are no mercury residue effects and that the system is stabilized. Click the "magic wand" icon on the bottom left of the screen to add sample lines. Under the sample name column, enter "eb" for empty boat. For blank analyses 0.2g is manually entered in the weight column. Confirm that the correct calibration and method are being used by selecting the **Links** tab. Switch back to the **Sample** tab and press the green **Start** button on the bottom right of the screen. Sample weights can be entered manually or automatically by the balance interface with the DMA-80.

12.2.1 Result values should be < ½ MRL. If they are not, run more empty boats. Choose the **Result** tab to view results.

12.3 Run low level CCV. Place a clean quartz boat on the scale, tare the scale, and weigh out 0.04g ± 0.004g of DORM-3 into the boat. Enter the weight in the DMA. Place the boat in the DMA-80 in the appropriate "POS" slot (number not in parenthesis). Enter this as batch number-SRM1 (Example: "P104035-SRM1") into the sample name column.

12.2.1 Note: When not actually running, the sample tray will always index to the front center position represented by the highlighted sample line on the screen. An arrow was marked on the instrument chassis to indicate this position.

12.2.2 A Remarks Column is available in this window for any individual comments for each sample line.

12.3 Run high level CCV. Weigh out 0.2g ± 0.02g of DORM-3 into the boat, enter this as batch number-SRM2 (Example: "P104035-SRM2").

12.3 Run CCB. Place an empty boat in the next slot and add as batch number-BLK1 (Example: "P104035-BLK1").

12.2.1 Verify CCV's and CCB are within range (see Table 3). If they are not, run another CCV/CCB set.

12.3 Run samples. Weigh out 0.2g ± 0.02g of homogenized fish tissue into a tared quartz boat. Enter the weight and sample number into the DMA-80. Wipe spatula with a Kimwipe between each sample. See Table 3 for example sample queue with QC guidelines.

12.2.1 If there is difficulty meeting the QA requirements, the instrument will require recalibration and/or replacement of the catalyst/amalgamator system and maintenance.

12.3 Quartz Boat Cleaning

12.2.1 Turn the muffle furnace on to 800˚C. The warm up usually takes about 45 minutes.

12.2.2 Remove any ash in boats by running Milli-Q water and scrubbing with a small test tube brush, taking care not to crack the boat. Rinse the boats thoroughly and place them face down on dry paper towels. Allow the boats to air dry or place the ceramic bowl and boats into a drying oven for 30 minutes or until dry at 105-110°C.

12.2.3 When the muffle furnace is up to temperature, place the ceramic bowl and quartz boats in the furnace using the long handled tongs (46 cm long), leave in the furnace for about an hour. Remove the porcelain dish using the tongs and carefully pour the quartz boats onto a sheet of asbestos for cooling, set bowl on asbestos as well. Let cool for at least 15 minutes, return boats to ceramic bowl and cover container to keep dust out.

12.3 Instrument Maintenance

12.2.1 Ensure the low pressure side gauge is at 60 psi and the high pressure is above 500 psi. When the high pressure gets down to 500 psi, the tank is empty. Replace it with a full oxygen tank.

12.2.2 Ensure all heaters are ready before starting an analysis. Click the **System** tab on the screen and check for the 3 OK labels at the bottom of the schematic.

12.2.3 Keep the autosampler tray clean. Wipe up spills immediately.

12.2.4 Maintain a complete and current maintenance log.

12.2.5 During routine maintenance of the DMA-80, both the unit and the lab terminal should be turned off and disconnected from the power supply. The oxygen supply must be turned off at the tank. Change the internal consumables only after the unit has cooled to room temperature.

13.0 Data Analysis and Calculations

13.3 When run is complete, click the “diskette” icon to save the results. Click “yes” to overwrite data. Log out and eject the Transcend memory card from the top of the DMA-80 monitor. Place memory card in easy-doc reader, and plug into a PC loaded with easy doc software.

13.3 The folder should automatically open, select the correct file and open it. If it does not open automatically, open the driver called "PIO" under My Computer, open the "Data" folder, and select the correct file. Click the **Print/Export** button to print the Results Page.

13.3 Click the **green X** (excel) icon. Save the file onto the G-drive exactly as the DMA file name was saved in *G:\EH\Eh-Lab\DataCapture\DMA_DataFiles\DMA-80\New DMA-80\(year)*

Leave excel file open, minimize.

13.3 Click the "diskette" icon and save a copy of the text file to the G-drive in *G:\EH\Eh-Lab\Data Capture\DMA_DataFiles\DMA-80\New DMA-80\Text\Text(year).* Close file. Safely remove the easy doc from the PC and return the memory card to the DMA-80 monitor.

13.3 Open the Element Database and go into the bench sheet of the batch to be reported. Click **Export** and save into "HgWeights". Replace file. C*:\ELMNT\UserFiles\ Hgweights.*

Open the "Hgweights" file. Using the Excel file created earlier from the DMA results, copy and paste the values from the weight column (J), in the DMA file to the "Hgweights" file. Column J weights must be copied into Column G (initial weight) and Column H (final weight) of the "Hgweights" file. The initial and final weights must match. Click on **Save** to save the "Hgweights" file.

Return to element database and re-open the batch's bench sheet. Select **Open** at the bottom of the window and open the "Hgweights" file. The new weights should upload into the bench sheet.

13.3 Highlight all samples, right click and change status from "batched" to "analyzed." While all samples are highlighted, right click, select "Prepared" and enter the date and time the DMA began analyzing. Be sure the analyst's initials are correct (Prepared by). Do the same for the QC's. Right click each SRM separately, select "Spike amount", multiply SRM weight x 1000 (ie. 0.2003g = 200.3) Save. Print the Bench Sheet.

13.3 Under the main Element window, click on "Laboratory" and select **Data Entry/Review.** An "Enter/Edit Data" window will appear. Click on the batch number. Select the **Data Entry** tab then **Create** to upload the data into the window below. Select the **Data Tool** button.

13.3 Select the correct file and double click on it; the samples will appear in the upper left window. Highlight the samples and QC's run, click **Include**, and select **Done**. (There will be a sample file added to the end of the list by Element that was not run. Do not include this file.) **Merge** the files.

13.3 Verify that the number of samples on the **Merge Upload** tab and the **Instrument Data** tab correlate. Rename any samples that are not named as they are in the Element batch under the **Instrument Data** tab. Click the **Refresh** button. Click on **Save**, choose the Element folder *C:\ELMNT\UserFiles,* and select the "gg" spreadsheet. Answer "Yes" to replace the existing data. Click **Done** and close the Data Tool window.

13.3 In the **Enter/Edit Data window**, change the analyst initials from ZZZ to the analyst's initials by clicking the Analyst column header, right-clicking, and selecting "Fill cells." Use the drop-down menu to select the analyst's initials.

13.3 Click **Save** in the Enter/Edit Data window to save the merged files. Click done. Under the Data Review tab select **Query.**

13.3 The test results will now appear. Lock the page by clicking on the Lock column header and selecting Lock. Click the Printer icon to print the Data Review report.

13.3 Calculations

13.2.1 Sample Concentration

$$\text{Concentration (mg/kg)} = \frac{\text{Mercury (Hg) (ng)}}{\text{Sample Weight (g)}} \times \frac{1000\text{g}}{1\text{kg}} \times \frac{1\mu\text{g}}{1000\text{ng}} \times \frac{1\text{mg}}{1000\mu\text{g}}$$

or

$$\text{Concentration (mg/kg)} = \frac{\text{Mercury (Hg) (ng)}}{\text{Sample Weight (g)}} \times \frac{1\text{mg}}{1000\text{kg}}$$

13.2.2 Percent Drift (%D)

$$\text{Drift (\%)} = \frac{\left|C_c - C_t\right|}{C_t} \times 100\%$$

where:
C_c= Calculated concentration
C_t= Theoretical concentration

12.13.3 Duplicate Relative Percent Difference (RPD)

$$\text{RPD} = \frac{\left|X_1 - X_2\right|}{\left(X_1 - X_2\right)/2} \times 100\%$$

where:
X_1 = Concentration of sample
X_2 = Concentration of duplicate

Significant figures: final results are rounded using EPA rules to 2 significant figures.

14.0 Data and Records Management

14.3 Assemble a data package consisting (in order):

14.2.1 Element bench sheet with uploaded weights.

14.2.2 Results report from element database.

14.2.3 Completed data review checklist.

14.2.4 Non-conformance details report (if applicable).

14.2.5 Calibration curve copy/absorbance vs. time graphic plots.

14.2.6 DMA-80 run log.

14.3 Place data package in an end tab manila folder with a label denoting the date of analysis *G:\EH\Eh-Lab\Chemistry\Label Files\AnalysisFolders\EPA 7473.LWL* and give completed file to chemistry supervisor for review.

15.0 Quality Control

15.3 QC requirements are summarized in Table 5.

15.3 IDC/MDL Studies: A set of 4 passing IDC's must be conducted by each new analyst; these are low-level CCV's. An MDL study must be conducted annually. If appropriate, the IDC and MDL studies may be combined.

15.2.1 An MDL study is conducted annually and/or when significant changes in instrument occur, or when a new instrument is purchased for the analysis.

15.3 Reporting Limit (RL): Reference Element for the current MRL for mercury in fish and shellfish tissue.

15.3 The R^2 of the calibration curve must be greater than or equal to 0.990.

15.3 Initial Calibration Verification (ICV): The ICV must be obtained from a second source other than that of the calibration standard. The DORM-3 CRM is used as the ICV and analyzed after calibration.

15.2.1 The ICV acceptance criterion is +/-10%. If recovery falls out, one rerun is allowed. If recovery remains outside of the allowable range, recalibration is required.

15.2.2 The DMA-80 has two cells in series for testing low and high level Hg. The low range cell can measure Hg from 0 to 35 ng and the high range cell can test Hg from 35 to 600 ng. After calibration, both low and high ranges ICVs are required to run.

15.2.3 If samples contain mercury concentration higher than the low range cell limit, then the high range cell ICV must be run with same acceptance criterion. Otherwise, only the low range cell ICV is required.

15.3 Continuing Calibration Verification (CCV): The DORM-3 certified reference material (CRM) is used as working CCV and analyzed at the beginning of a run, after every 10 samples and at the end of the run.

15.2.1 The initial CCV acceptance criterion is +/-10%. The following pairs of CCV's have an acceptance criterion of +/-20%. If recovery falls out, one rerun is allowed. If recovery remains unacceptable, recalibration is required with rerun of all samples analyzed after last acceptable CCV.

15.2.2 If samples contain mercury concentration higher than the low range cell limit, then the high range cell CCVs must be run with same acceptance criterion. Otherwise, only the low range cell CCVs are required.

15.3 Method Blank/Calibration Blank (MB/CB): A calibration blank must be analyzed after each pair of CCV's.

15.2.1 The level of total Hg in the MB must be ≤ ½MRL.

15.2.2 When MB/CB contamination is apparent, samples associated with the MB/CB may require a rerun.

15.2.3 If the measured sample concentration is greater than 10 times the MB/CB contamination, the sample does not need reanalysis. If the sample has a concentration < MRL, the sample does not need to be rerun. All other associated samples will require reanalysis.

15.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD). An MS/MSD pair is performed with every sample batch of 20 samples or less, or at the frequency requested by the client.

15.2.1 The MS/MSD aliquots are spiked with calibration standard as described in section 10.4.

15.2.2 The acceptance limits are 80%-120% recovery.

15.2.3 If the spiking value is less the 5x the concentration of native concentration of Hg in the sample, the acceptance limits are advisory only.

15.2.4 If recoveries are outside acceptance limits then the data is flagged as estimated (J qualifier).

15.3 Sample Duplicate: A sample duplicate is performed with every sample batch of 20 samples or less, or at the frequency requested by the client. One duplicate for every 10 samples are run for fish tissue.

15.2.1 The advisory RPD for a sample duplicate is ≤ 20%.

15.2.2 A reanalysis will be required when the allowable Relative Percent Difference (RPD) limit is exceeded. If the rerun batch has a RPD > 20%, the sample will be flagged as non-homogeneous.

16.0 References

16.3 USEPA, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW8-46, 3rd Edition; Method 7473.

16.3 "*Guidance for Preparing Standard Operating Procedures (SOPs)*", United States Environmental Protection Agency, EPA QA/G-6, March 2001.

16.3 Milestone DMA-80 Direct Mercury Analyzer User Manual – Revision 3/2004

16.3 EPA 7473, Revision 0, February 2007.

Table 1
Temperature Program for DMA-80

Pos	t	T
1	00:01:00	200
2	00:03:00	650

Table 2
Analytical Parameters for DMA-80

Parameter	Value
Max Start T	200°C
Purge time	60s
Amalgamator heating time	12s
Signal recording time	30s

Table 3
Example Sample Queue

Sample Name	Quality Control	Notes
Element batch#-SRM1 (ie. P103006-SRM1)	Low level Initial Calibration Verification (ICV)	0.3438-0.4202 mg/kg (±10% of 0.382 true value)
Element batch#-SRM2	High level Initial Calibration Verification (ICV)	0.3438-0.4202 mg/kg (±10% of 0.382 true value)
Element batch#-BLK1	Initial Calibration Blank (ICB)	< ½ MRL
Batch Samples 1-10	Element sample number (*ie. 1010003-40*)	Each set of 10 must have a sample duplicate.
Element batch#-DUP1	First sample duplicate	Randomly selected sample is measured and run again.
Element batch#-MS1	Matrix Spike (MS) 80-120% recovery	Same sample source as DUP1 or DUP2; but run w/ 50µL spike
Element batch#-MSD1	Matrix Spike Duplicate (MSD) 80-120% recovery	Same sample source as DUP1 or DUP2; but run w/ 50µL spike
Element batch#-SRM3	Low level Continuing Calibration Verification (CCV)	0.3056-0.4584 mg/kg (±20% of 0.382 true value)
Element batch#-SRM4	High level Continuing Calibration Verification (CCV)	0.3056-0.4584 mg/kg (±20% of 0.382 true value)
Element batch#-BLK2	Continuing Calibration Blank (CCB)	< ½ MRL
Batch Samples 11-20	Element sample number	Each set of 10 must have a sample duplicate.
Element batch#-DUP2	Second sample duplicate	Another randomly selected sample is measured and run again.
Element batch#-SRM5	Low level Continuing Calibration Verification (CCV)	0.3056-0.4584 mg/kg (±20% of 0.382 true value)
Element batch#-SRM6	High level Continuing Calibration Verification (CCV)	0.3056-0.4584 mg/kg (±20% of 0.382 true value)
Element batch#-BLK3	Continuing Calibration Blank (CCB)	< ½ MRL

Table 4
Mercury (Hg) Standard Volumes for Calibration Curve

Name	Standards Size	ng of Hg	Software Weight Entry	Software Concentration Entry
ICAL 1	20µl 1% Nitric Acid	0ng	0.0001	0.0001

ICAL 2	10µl 0.1mg/L Mercury (Hg) Working Calibration Standard	1ng	0.01	0.1
ICAL 3	20µl 0.1mg/L Mercury (Hg) Working Calibration Standard	2ng	0.02	0.1
ICAL 4	50µl 0.1mg/L Mercury (Hg) Working Calibration Standard	5ng	0.05	0.1
ICAL 5	10µl 1.0mg/L Mercury (Hg) Working Calibration Standard	10ng	0.01	1
ICAL 6	15µl 1.0mg/L Mercury (Hg) Working Calibration Standard	15ng	0.015	1
ICAL 7	20µl 1.0mg/L Mercury (Hg) Working Calibration Standard	20ng	0.02	1
ICAL 8	50µl 1.0mg/L Mercury (Hg) Working Calibration Standard	50ng	0.05	1
ICAL 9	10µl 10mg/L Mercury (Hg) Working Calibration Standard	100ng	0.01	10
ICAL 10	20µl 10mg/L Mercury (Hg) Working Calibration Standard	200ng	0.02	10
ICAL 11	50µl 10mg/L Mercury (Hg) Working Calibration Standard	500ng	0.05	10

Table 5
Quality Control Requirements

Parameter	Frequency	Acceptance Criteria	Corrective Action
Initial Calibration	As required	5 points minimum for both low and high range. $R^2 \geq 0.990$	Rerun initial calibration
Initial Calibration Verification (ICV)	At beginning of sequence and every 10 samples	ICV ±10% of the true value	Rerun once. If ICV still out after re-analysis then re-calibrate.
Continuing Calibration Verification (CCV)	After every 10 samples	CCVs ±20% of the true value	Rerun once. Re-analyze samples analyzed after the last acceptable CCV. If CCV still out after re-analysis then re-calibrate.
Method Blank (MB)	Once per batch of 20 samples or daily, whichever frequency is greater.	< ½ MRL	Rerun once. Investigate source of contamination. Re-analyze associated samples if results are > RL or < 10X MB level.
Matrix Spike/ Matrix Spike Duplicate (MS/MSD)	Once per batch of 20 samples or daily, whichever frequency is greater.	Recovery ±20%	Rerun once. If MS/MSD recovery is >120% and samples are >RL, then report results with J qualifier.
Sample Duplicate (DUP)	Once per batch of 20 samples or daily, whichever frequency is greater.	RPD ≤ 20%	Rerun once. If RPD still not within acceptance criteria qualify sample results.

Table 7
Data Review Checklist – EPA Method 7473

Date of Analysis: _____ Project Name: _____

Calibration			
Review Item	**Analyst**	**Reviewer**	**Comments**
1. Initial Calibration performed at high and low level, with at least 3 points per level?			
2. Initial Calibration $r^2 \geq 0.990$ for high and low level?			
Batch QC			
Review Item	**Analyst**	**Reviewer**	**Comments**
1. Initial Calibration Verification (ICV) within ±10% of the true value?			

2. CCV every 10 samples and closing?			
3. Continuing Calibration Blank (CCB) after every CCV?			
4. CCB ≤ ½MRL?			
5. Closing CCV within ±20% of the true value?			
6. MS/MSD pair analyzed every 20 samples?			
7. MS/MSD recovery 80%-120%?			
8. Samples duplicate every 10 samples?			
9. Sample duplicate RPD ≤ 20%			
Samples			
Review Item	**Analyst**	**Reviewer**	**Comments**
1. Results within calibration range?			
2. Data entry spot checked at least 10%?			

Reviewer_____ Date:_____

Table 8
Catalyst Tube Conditioning Procedure

Introduction:
Every time a new catalyst or amalgamater tube is replaced a new calibration curve is required, but at first the following conditioning procedure must be followed.

This process is not necessary for DMA80 units delivered directly from Milestone because this was already done during final quality control testing.

Note: Before starting the procedure, be sure that the catalyst/amalgamator are correctly installed and the DMA80 unit is ready to operate (alignment of actuators, stability of Oxygen flow). For more details see the operator manual.

DMA80 procedure for conditioning new catalyst/amalgamator tubes:

Step	Reagent/Sample Type	Sample Amount	DMA80 Program		
			Drying Time and Temperature(1)(2)	Decomposition Time and Temperature	Purge/Waiting Time
1	Common white flour	100mg	IR: 60sec/300ºC	IR: 180sec/850ºC	60sec
			ATC: 60sec/200ºC	ATC:180sec/650ºC	
2	HNO_3, 5%(3)	0.1mL	IR: 60sec/300ºC	IR: 180sec/850ºC	60sec
			ATC: 60sec/200ºC	ATC: 180sec/650ºC	
(1) The program has to be set according to the type of temperature control on the drying/decomposition furnace: external infrared (IR) or internal thermocouple (ATC) sensor.					
(2) In case of T640/T1640 controller the reported temperature and time refer to the "holding" step.					
(3) Quartz sample boat required. In case of metal boat usage, they must be deeply cleaned and a stability test run.					

Repeat the above procedure at least five times, or until the blank absorbance is <0.0030.

After condition a stability test must be run. Analyze a fresh aqueous 100ppb standard five times. The RSD must be <1.5%. For this analysis the recovery is not as important as the DMA stability.

	Signature and Title	**Date**
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This is a controlled document with the most recent updated revision. This SOP should be reviewed on an annual basis. If the SOP is found adequate, the SOP cover page is signed and dated for documenting the review. If major revisions are needed, a new revision will be released with a new signature page. The above signatures reflect periodic review of the Standard Operating Procedure.

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1.0. OBJECTIVE:

This procedure describes the laboratory fish tissue processing procedures for the assessment of contaminant levels in a variety of marine and freshwater fishes of Alaska. Fish tissue samples are prepared in accordance with U.S. Environmental Protection Agency (EPA) guidance (U.S. EPA 2000). Sample processing and homogenization of tissues are performed at the DEC Environmental Health (EH) Lab. The Project Coordinator oversees fish sample processing activities.

2.0. SCOPE AND APPLICATION:

Processing for most fish samples consists of removal of both fillets from each fish, removal of the skin from both fillets, homogenization of the entire amount of tissue from both fillets, and apportionment of the homogenized tissue from each fish into two to five pre-cleaned 4 oz. glass sample jars, depending on whether the sample was chosen for basic analysis (2 jars), or organochlorine compound analysis in addition to the basic metals analysis (3-5 jars). Exceptions to this are for species such as Pacific halibut (*Hippoglossus stenolepis*), where a roast removed from one fillet of a fish has been received from the field sampler, or dockside and similar samples where a small amount of tissue has already been removed from a fish in the field and is shipped to the lab in a zip-lock baggie. For halibut, the skin is removed along with a layer from each side of the roast. The roast is then homogenized following the same procedures used for fillets. For dockside and similar programs, the outer surfaces of the sample are removed.

3.0. INTERFERENCES:

3.1. Stainless steel knives, blades and blenders are used to minimize contamination of the samples by heavy metals.

4.0. HEALTH AND SAFETY:

4.1. Before performing this SOP, the pertinent MSDS for each chemical should be read.

4.2. Gloves, eye protection glasses and lab coat should be worn at all times while performing this analysis.

4.3. Knives should be kept sharp. Whenever possible, cuts should be made away from the body. A Kevlar glove should be worn to protect the non-knife hand.

5.0. SAMPLE COLLECTION, PRESERVATION, AND STORAGE:

5.1. Biologists from IPHC, ADF&G, NOAA, other agencies, and commercial and native fishermen have collected whole fish or roast samples from predetermined locations. These samples are sent to the EH Lab frozen.

5.2. Fish samples are stored at -20°C until they are ready to be thawed for processing.

5.3. Fish samples are thawed at 4°C. Fish should be thawed only to the point at which it becomes possible to safely make the necessary cuts (U.S. EPA 1990).

5.4. Processed fish tissue samples are returned to the freezer and stored in wide mouth jars at -20°C until analysis.

6.0. EQUIPMENT AND SUPPLIES:

6.1. Lab coat and safety glasses.

6.2. Analytical balance for use in weighing samples.

6.3. Laboratory dishwasher.

6.4. Commercial tissue grinder.

6.5. Fume hood.

6.6. Kevlar glove.

6.7. Nitrile gloves for handling fish.

6.8. Cutting boards.

6.9. Fillet knives.

6.10. Chemical and biological waste storage containers.

6.11. Labware:

6.11.1. Glassware – pre-cleaned I-Chem® 4 oz wide mouth jars with lids. (Processed (PC) and Quality-Assured® (QA) bottles meet EPA specifications. QA bottles are supplied with a Certificate of Analysis. PC and QA cases are custody-sealed to ensure reliable chain-of-custody.)

6.11.2. Foil.

6.11.3. Teflon spatula.

6.11.4. Marked Teflon squeeze bottles for DI water, dilute nitric acid, acetone, cyclohexane, and methylene chloride.

7.0 REAGENTS AND STANDARDS:

7.1 Nitric Acid (metals grade)

7.2 Acetone (pesticide grade).

7.3 Cyclohexane (pesticide grade).

7.4 Methylene chloride (pesticide grade).

7.5 DI Water (RO filtered).

8.0 PROCEDURE

8.1. Inspection:

8.1.1. *Individual fish received are unwrapped and carefully inspected to ensure that they have not been compromised in any way e.g. decomposed, not properly preserved during shipment, too small for individual processing, arrived with incomplete or missing Field Data Collection form.*

8.1.2. Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing sheet.

8.1.3. All fish are inspected for lesions or other indications that they are not healthy.

8.1.4. The fish processing technician immediately informs the project coordinator of any unsuitable or unhealthy samples. Lesions and indications found are documented on the sample processing sheet.

8.2. Sample Weighing:

8.2.1. Prior to processing each fish, the Technician washes their hands with soap and rinses thoroughly in tap water, followed by a distilled water rinse (U.S. EPA 1990).

8.2.2. Fresh nitrile gloves are worn for each fish processed. For safety, a Kevlar glove is worn under the glove of the non-knife hand.

8.2.3. A wet weight is determined for each fish in the processing laboratory.

8.2.4. Samples are weighed on a properly calibrated scale with adequate accuracy and precision (±1 gram).

8.2.5. Fish are placed on a foil-lined balance tray. To prevent cross contamination between individual fish, the foil lining is replaced after each weighing.

8.2.6. All weights are recorded to the nearest 10 grams on the sample processing sheet.

8.3. Sex Determination:

8.3.1. To determine the sex of a fish, an incision is made on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the isthmus. If necessary, a second incision is made on the left side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap is folded back to more easily observe identifiable reproductive tissue.

8.3.2. The sample identification number and sex of each fish is recorded on the sample processing sheet.

8.3.3. If unsure of the sex of any sample, the project coordinator is contacted for verification before further processing of the fish.

8.3.4. If the fillet tissue becomes contaminated by puncture of the internal organs during resection, all contaminated tissue will be cut away and discarded. A notation is made describing the event and its severity on the sample processing sheet.

8.4. Filleting:

8.4.1. Filleting is conducted under guidance of the project coordinator or other experienced biologist.

8.4.2. Fish are filleted on a plastic cutting board that is properly cleaned and decontaminated between fish (see below for cleaning procedure).

8.4.3. Care is taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs.

8.4.4. Clean, decontaminated, high-quality stainless steel utensils are used to remove both fillets from each fish, as necessary.

8.4.5. A shallow cut is made through the skin, on either side of and along the dorsal fin, from the base of the skull to the base of the tail.

8.4.6. Careful vertical cuts are made behind the gill cover through the skin and flesh to the spine. The length of the cut is from the dorsal to ventral surface of the fish.

8.4.7. The fillet is then removed by cutting along the spine and over the ribs from front to back.

8.4.8. The belly flap is included with each fillet, but the pelvic fins, if present, are removed.

8.4.9. When the fillet remains connected to the fish only at the tail, the fillet is removed from the skin, leaving the skin attached to the fish carcass.

8.4.10. Bones still present in the tissue after filleting are removed carefully (U.S. EPA 1990).

8.4.11. Both fillets are removed from a fish.

8.4.12. Both fillets from a fish, with belly flesh attached, are homogenized as a sample.

8.4.13. **Roast and small tissue samples** are processed by removing the skin, if present, and cutting away the outer layer of tissue from all sides of the roast. This is done to remove any tissue that may have come into contact with contaminants. The roast is then treated the same as the pair of fillets from other fish species.

8.5. Preparation of Individual Homogenates:

8.5.1. To ensure even distribution of contaminants throughout tissue samples, fillets are ground and homogenized prior to analyses.

8.5.2. Fillets are ground in a tissue grinder or are minced by hand with the fillet knife used to remove the fillets. For larger samples a commercial tissue grinder is used. The grinder is made of aluminum and stainless steel. As grinding and homogenization of biological tissue is easier when the tissue is partially frozen (Stober 1991), samples are homogenized immediately after removal from the carcass.

8.5.3. For standard size tissue samples, tissues are run through the grinder at least three times. If there is still un-macerated tissue in the sample, additional grinding is performed.

8.5.4. If only small amounts of tissue are available, the sample is trimmed to remove possible contaminants, and is then minced to the smallest size possible with the fillet knife. The resulting tissue should be roughly comparable to the grinder homogenate

8.5.5. A Teflon spatula is used to ensure that all tissue is continually being run through the grinder blades.

8.5.6. Homogenization of each individual fish is noted on the sample processing sheet.

8.5.7. Homogenates from each fish are apportioned into two to five pre-cleaned labeled 4 oz. jars, depending on needs for analysis, frozen and stored at -20°C. Analysis decisions are made by the program manager or project coordinator.

8.5.8. Each homogenate portion size is roughly three ounces. The jar should not be completely filled.

8.6. Decontamination Procedures Between Samples:

8.6.1. The cleaning and preparation of all equipment prior to processing fish tissue ensures no cross contamination between samples.

8.6.2. All knives, cutting boards and grinder parts are rinsed in the lab sink with de-ionized water and then either washed in an approved laboratory dishwasher with a detergent, followed by a mild acid rinse, and a de-ionized water rinse, or hand washed with liquinox and deionized water. If hand washing is used, all equipment should be machine washed at the end of the day.

8.6.3. If only a small amount of tissue is available, and the sample is minced with a fillet knife, the knives and cutting board may be washed by hand in the sink instead of running them through the dishwasher.

8.6.4. If tissues will or may be used in organochlorine compound analysis, all equipment is then triple-rinsed with each of the following: acetone, cyclohexane, and methylene chloride. All parts are then air dried.

8.6.5. If the tissues will be analyzed only for metals, then all equipment is rinsed in 1% (v/v) nitric acid, rinsed in ultra-pure water, and allowed to air dry.

8.6.6. Organic (acetone and cyclohexane) and chlorinated (methylene chloride) chemical waste are disposed of in a secondary hazardous waste container under the fume hood.

9.0 QUALITY CONTROL:

9.1 In order to maintain high quality homogenates, the following will be maintained:

9.1.1 The technician is properly trained in knife handling and filleting, to protect the quality of the tissue during removal.

9.1.2 All data is immediately entered onto the fish processing sheets as it is collected.

9.1.3 To ensure that there is no contamination of the solvents, squeeze bottles are filled one type at a time. Solvents are never returned to their original container after being placed in the squeeze bottles.

10.0 DATA HANDLING:

12.1 Data is entered into the SQL Server by the technician. The project coordinator checks all data entered and maintains records of all data quality checks.

11.0 INSTRUMENTATION MAINTENANCE:

11.1 The scale is calibrated before initiating the processing, and is re-calibrated annually.

12.0 REFERENCES

12.1 Stober, Q. J. 1991. Guidelines for Fish Sampling and Tissue Preparation for Bioaccumulative Contaminants. Environmental Services Division, Region 4, U.S. Environmental Protection Agency, Athens, GA.

12.2 Texas Water Commission. 1990. Texas Tissue Sampling Guidelines. Texas Water Commission, Austin, TX.

12.3 U.S. EPA. 2000. Guidance for assessing chemical contaminant data for use in fish advisories, Volume 1 - Fish sampling and analysis. Third Edition. EPA 823-B-00-007. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

STANDARD OPERATING PROCEDURE
DETERMINATION OF TRACE METALS IN WATERS AND WASTES BY EPA 6020A

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5.1.2 **Page**

17.0 Scope and Application

1.1 Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of a large number of elements in water samples and in waste extracts or digests. When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, fish tissue and other solid wastes for which total elements are required. If Method 6020A is used to determine any analyte not listed in this SOP, it is the responsibility of the analyst to demonstrate the accuracy and precision of the method in the sample matrix to be analyzed. The analyst is always required to monitor potential sources of interferences and take appropriate action to ensure data of known quality. Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices. Use of this method should be relegated to spectroscopists who are knowledgeable in the recognition and in the correction of spectral, chemical, and physical interferences in ICP-MS.

2.0 Summary of Method

2.1 A 0.5g to 1.0 gram portion of sample (fish tissue or soil) is digested with concentrated HNO_3 and refluxed until no brown fumes are given off indicating the complete reaction with HNO_3. Water and 30% H_2O_2 are then added and the refluxing is continued until the effervescence subsides. Heating then continues for an additional 2 hours after which the sample is brought up to a final volume with water and the sample analyzed for trace metals individually detected and quantified by ICP-MS.

2.0 Health and Safety Warnings

3.1 Before performing this SOP, the pertinent MSDS for each chemical should be read.

3.2 Gloves, eye protection glasses and lab coat should be worn at all times while performing sample extraction steps, or when handling samples or extracts

3.3 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. All expired stock and working standards must be disposed of by current ADEC EHL protocol.

3.4 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

3.5 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

3.6 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection for UV emissions.

4.0 Cautions

4.1 During digestion, do not allow the sample to boil or evaporate to dryness. Keep sample covered with a watch glass while refluxing to avoid analyte loss.

5.0 Interferences

5.1 Isobaric elemental interferences - Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal mass-to-charge ratio. A data system must be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal. Since commercial ICP-MS instruments nominally provide unit resolution at 10% of the peak height, very high ion currents at adjacent masses can also contribute to ion signals at the mass of interest. Although this type of interference is uncommon, it is not easily corrected, and samples exhibiting a significant problem of this type could require resolution improvement, matrix separation, or analysis using another verified and documented isotope, or use of another method.

5.2 Isobaric polyatomic ion interferences - Isobaric molecular and doubly-charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature (References 3 and 4). Examples include ArCl ion on the As signal and MoO ions on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotope abundances from the literature (Reference 5), the most precise coefficients for an instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting statistics. Because the ^{35}Cl natural abundance of 75.77 percent is 3.13 times the ^{37}Cl abundance of 24.23 percent, the chloride correction for arsenic can be calculated (approximately) as follows (where the $^{38}Ar^{37}Cl^{+}$ contribution at m/z 75 is a negligible 0.06 percent of the $^{40}Ar^{35}Cl^{+}$ signal):

5.2.1 Corrected arsenic signal (using natural isotopes abundances for coefficient approximations) = (m/z 75 signal) - (3.13) (m/z 77 signal) + (2.73) (m/z 82 signal), where the final term adjusts for any selenium contribution at 77 m/z, NOTE: Arsenic values can be biased high by this type of equation when the net signal at m/z 82+ 81+82 is caused by ions other than Se, (e.g., BrH from bromine wastes [Reference 6]).

5.2.2 Corrected cadmium signal (using natural isotopes abundances for coefficient approximations) = (m/z 114 signal) - (0.027)(m/z 118 signal) - (1.63)(m/z 108 signal), where last 2 terms adjust for any $^{114}Sn^{+}$ or $^{114}MoO^{+}$ contributions at m/z

114. NOTE: Cadmium values will be biased low by this type of equation when $^{92}ZrO^{+}$ ions contribute at m/z 108, but use of m/z 111 for Cd is even subject to direct ($^{94}ZrOH$) and indirect (^{90}ZrO) additive interferences when Zr is present. NOTE: As for the arsenic equation above, the coefficients could be improved. The most appropriate coefficients for a particular instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting precision. The accuracy of these types of equations is based upon the constancy of the OBSERVED isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found (Ref. 7) to be reliable, e.g., oxide levels can vary with operating conditions. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferent. For example, this type of correction has been reported (Ref. 7) for oxide-ion corrections using ThO /Th for the determination of rare earth elements. The use of aerosol desolvation and/or mixed gas plasmas have been shown to greatly reduce molecular interferences (Ref. 8).

5.3 Physical interferences – Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement. Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Total solid levels below 0.2% (2,000 mg/L) have been currently recommended (Ref. 10) to minimize solid deposition. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes (Ref. 11). When intolerable physical interferences are present in a sample, a significant suppression of the internal standard signals (to less than 30% of the signals in the calibrations standard) will be observed. Dilution of the sample fivefold (1+4) will usually eliminate the problem. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects. Internal standards ideally should have similar analytical behavior to the elements being determined.

5.4 Memory interferences - Memory interferences or carry-over can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

6.0 Personnel Qualifications

6.1 This procedure may only be performed by an EHL Laboratory Technician or Chemist I-IV. Analysts independently performing this method must complete an Initial Demonstration of Capability. Analysts who have not completed an Initial Demonstration of Capability may perform the method only under the direct supervision of the Chemistry Supervisor or other qualified staff.

7.0 Sample Collection, Handling and Preservation

7.1 Fish Tissue: For the determination of elements in fish tissues, biologists from International Pacific Halibut Commission (IPHC), Alaska Department of Fish and Game (ADF&G), Nation Oceanic and Atmospheric Administration (NOAA), and commercial and native fisher-men or others will collect samples from predetermined locations. Skinless fillets are homogenized. The homogenized tissue is stored in wide mouth jars in the freezer at ≤ -10°C until the day before analysis. Then it is thawed in the refrigerator until it is weighed out. The unused portion is returned to the freezer.

7.2 Water samples: For the determination of total recoverable elements in aqueous samples, samples are not filtered. The samples are acidified with nitric acid to pH <2 (or are received already acidified). The typical sample size is 1L. The samples are stored at room temperature.

7.3 Soil and other solid samples: For the determination of total recoverable elements in solid samples, samples are stored at 4ºC. The typical sample size is 50g, but may be smaller.

7.4 Extracts are stored at 4°C for at least 30 days, or until such time as it is determined that no further analysis be needed. Once confirmed that no more analysis will be needed the samples may be disposed.

8.0 Equipment and Supplies

8.1 PerkinElmer Elan ICP-MS DRC II.

8.2 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

8.3 Analytical balance, with capability to measure to 0.0001 g, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

8.4 A temperature adjustable hotblock capable of maintaining a temperature of 95°C.

8.5 A gravity convection drying oven with thermostatic control capable of maintaining 105°C ± 5°C

8.6 Air displacement pipetters capable of delivering an assortment of volumes ranging from 0.1-2500 µL

8.7 An assortment of high quality disposable pipet tips appropriate for the pipetters used.

8.8 Labware:

8.8.1 Glassware – volumetric flasks.

8.8.2 Environmental Express 68mL digestion cups with screw caps and watch glasses.

8.8.3 50mL and 15mL conical tubes.

8.8.4 Narrow-mouth storage bottles, plastic with screw closure.

8.8.5 One-piece stem plastic wash bottle with screw closure, 125 mL.

8.8.6 Squeeze bottle for DI (reagent) water.

9.0 Reagents and Standards

9.3 Reagent water - All references to reagent grade water in this SOP refer to ASTM Type I water (ASTM D1193).

9.4 Reagent Acids:

9.2.1 Nitric acid, Ultrex II Ultrapure Reagent HNO_3, Fisher Scientific.

9.2.2 Hydrochloric acid, Ultrex II Ultrapure Reagent HCl, Fisher Scientific.

9.2.3 Hydrogen peroxide (30%), Ultrex II Ultrapure Reagent H_2O_2, Fisher Scientific.

9.3 1% Nitric Acid (HNO_3) solution: add 20 mL concentrated HNO_3 solution to 2L DI (reagent) water. This is used for making the ICV, CCV, LCS, and calibration standards.

9.4 Standard Stock Solutions - Stock standards may be purchased from a reputable commercial source. Stock solutions should be stored in plastic (such as polyethylene) bottles. Replace stock standards before the expiration date.

9.5 Calibration Standards:

9.5.1 Intermediate Calibration Standard: Dilute the 100 mg/L stock standard AccuStandard (ICP-MS-200.8-Cal 1R) to make the calibration standards at 1, 10, 100, and 300µg/L.

9.6 Tuning Solution (6020TS –Inorganic Ventures): 1ppb Elan 6100DRC setup solution.

9.7 Daily Performance Check Solution (made by diluting Inorganic Ventures standards of Mg, In, U, Ce, and Ba): 1ppb Elan 6100 DRC sensitivity detection limit solution.

10.0 Instrument Setup, Calibration and Standardization

10.1 Power up the PerkinElmer Elan ICP-MS DRC II.

10.2 Turn on the argon supply at the tank and adjust the regulator to 50 psi.

20.3. Check that the sample tubing and drain tubing leading from the spray chamber are properly set up. Click on the instrument icon and then on the front panel button to open the ELAN control panel. The status indicator should indicate “ready” if all system hardware is operating properly and the plasma can be ignited. If the indicator says “not

ready", refer to the operator manual to determine which system component is causing the fault.

20.4 Click on the plasma start button to ignite the plasma. The ELAN initiates the ignition process which requires a little over a minute to complete. The progress of the ignition sequence is shown in the ignition sequence status bar.

10.5 After the plasma ignites, allow the instrument to warm up for approximately 15 minutes. If the plasma does not ignite, refer to the manuals to troubleshoot and correct the problem.

10.6 To perform a tuning check of the ICP-MS, open the EPA 6020A tuning method. Aspirate the tuning solution at the same time. Click the analyze sample button.

10.7 When the sample is done, check the report and make sure that the mass values are ±0.1 amu of the actual mass values and the resolution is less than 0.9 ± 0.1 amu.

10.8 If you have not yet reached the target resolution, open the optimization method for the optimization procedure. If you pass the tuning, click the EPA 6020A method, aspirate the daily performance check solution at the same time. Click the analyze sample button to run the daily check. When the measurement is complete, compare the result with table-1 to see if it falls within the ranges. If any value is outside the range, repeat the procedure again.

10.9 In the EPA 6020A workspace, open the "EPA 6020A quantitative analysis method". Enter a blank and six calibration standards on the calibration page. Select the appropriate (µg/g) unit and linear calibration type.

10.10 Press the sample icon; enter the autosampler locations for the blanks and standards. Enter the appropriate sample flush delay (typically 35s) and rinse (typically 45s) parameters. Type a new data name and the description, and save the new data file.

10.11 Place the calibration standard tubes into the positions indicated in EPA 6020A quantitative page.

10.12 Return to the main EPA 6020A quantitative page; Press the "Analyze Batch" icon to begin the calibration. Once finished running all the calibration points, click the "CalibView" icon. Select linear calibration algorithm with R ≥0.995.

10.13 If there is difficulty meeting the linear calibration algorithm with R ≥0.995, the instrument will require recalibration and/or maintenance.

11.0 Sample Preparation

11.1 All samples are prepared by EPA 200.8 or EPA 3050B.

11.2 Method Blank and Calibration Blank: fill 50 mL of 1% HNO_3 into a sample tube.

11.3 Continuing Calibration Verification Standard (CCV): Transfer 0.5 mL calibration stock into a 50mL sample tube and fill to volume with 1% HNO_3 to make a 100 µg/L CCV standard.

11.4 Laboratory Control Sample (LCS): Transfer 0.25ml calibration stock standard (Inorganic Ventures – WW-MSCAL-2) into a 50ml sample tube and fill to volume with 1% HNO_3 to make a 100µg/L LCS standard.

11.5 Matrix Spike/Matrix Spike Duplicate (MS/MSD) set: Spike the 10ml post-digestion MS/MSD samples with 50µL of the 20 mg/L stock standard. Spike the soil/fish tissue samples with 500µL of the 20mg/L stock standard. These spike volumes are suggested, other volumes may be used if appropriate.

12.0 Sample Analysis

12.1 Follow instrument warm up and set up procedure described in section 10.5.

12.2 Click the EPA6020A quantitative method to make it current. Open the sample file window and select batch mode. Select samples and click build run list. Verify that all the samples, standards and QC's are in the list along with the correct autosampler locations. Click on analyze batch to begin the run. Use the EPA 6020A quantitative calibration report to print the calibration report. When the run is complete, compare the results to the known value.

12.3 If there is difficulty meeting the QC requirements, the instrument will require recalibration and/or maintenance.

13.0 Data Analysis and Calculations

13.1 Calculations:

Sample Concentration

$$\text{Concentration } (\mu g/g) = \frac{(Df)(\text{ppm found})}{}$$

Where:
Df = Dilution factor. If no dilution was made, D = 1.

Percent Drift (%D)

$$\%\text{Drift} = \frac{|C_c - C_t|}{(C_t)} * 100\%$$

where:
C_c= Calculated concentration
C_t= Theoretical concentration

LCS Percent Recovery

$$\%R = \frac{(C_s)}{(C_a)} * 100$$

where:
C_s = Observed spike concentration
C_a = Spike Level

Duplicate Relative Percent Difference (RPD)

$$RPD = \frac{|X_1 - X_2| * 100}{(X_1 + X_2)/2}$$

where:
X_1 = Concentration of sample
X_2 = Concentration of duplicate

14.0 Data and Records Management

14.1 Enter analytical results into the EHL database. Enter final results in µg/g (use < flag for Non-detects, followed by the RL), method, analyzed by, analyzed date, report date. Significant figures: final results are rounded using EPA rules to 2 significant figures.

14.2 Print final results for the sample batch using the "Report" function in the EHL database.

14.3 Assemble a data package consisting (in order);

14.3.1 Final report from the EHL database
14.3.2 Data Review Checklist
14.3.3 Non Conformance Details report(s) (if applicable).
14.3.4 Sample Preparation Bench Sheet.
14.3.5 PerkinElmer ICP-MS Elan DRC II run log.
14.3.6 Calibration curve
14.3.7 Elan DRC II EPA 6020A Qualitative Analysis Summary Report for each sample and QC sample.
14.3.8 Place data package in an end tab manilla folder, with pre-printed label indicating the method performed and the date.

14.4 Submit data package to peer or supervisor for second level review. Upon successful review the second level reviewer enters their name and date into the EHL database.

14.5 Submit reviewed package to Chemistry Supervisor for final review. Chemistry supervisor or designee places data package to central files.

15.0 Quality Control

15.1 IDC/MDL Studies: An IDC must be conducted by each new analyst. An MDL study must be conducted annually. If appropriate, the IDC and MDL studies may be combined.

15.1.1 Four passing LCS's must be provided to validate an analyst for this method.

15.1.2 An MDL study should be conducted when significant changes in instrument occur, or when a new instrument is purchased for the analysis.

15.2 The R of the calibration curve must be greater than or equal to 0.995.

15.3 Precalibration routine: Analyze tuning solution 4 times to make sure the RSD of absolute signals <5%. Resolution at low mass is indicated by magnesium and resolution at high mass is indicated by lead. Adjust spectrometer resolution to produce a peak width of less than 0.9amu at 10% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu.

15.4 Internal Standards: The absolute response of any internal standard must be greater than 30% of the original response in the calibration blank, otherwise flush instrument with rinse blank and monitor response of the calibration blank. If the response for the calibration blank is within limits, dilute a fresh aliquot of sample 5x, add internal standard and rerun. If the response for the calibration blank is not within limits, terminate analysis and correct the problem.

15.5 EPA 6020A specific Criteria: All masses which might affect data quality must be monitored. Isobaric polyatomic ion interference must be recognized and appropriate corrections made to the data. Dilute and rerun samples that are more concentrated than the linear or measure an alternate less-abundant isotope.

15.5.1 Interference Check Solution should be run every 12 hours during active analysis of samples.

15.5.2 One Dilution test should be run every 20 samples. The result must be within 10% of the original measurement.

15.5.3 A post-digestion spike should be run if matrix interference is suspected in a sample. The result should be within 15% of the original measurement plus the spiked amount.

15.6 Initial Calibration Verification:

15.6.1 The ICV acceptance criterion is +/-10%.

15.6.2 The concentration of standards should range from 10µg/L to 200µg/L.

15.7 Continuing Calibration Verification (CCV): The 100µg/L is used as working CCV and analyzed at the beginning of a run, after every 10 samples and at the end of the run.

13.4.1 The CCV acceptance criterion is +/-10%. If the recovery is not within +/-10%, recalibrate and rerun all the samples since last compliant continuing calibration standard.

15.8 Method Blank/Calibration Blank (MB/CB): A calibration blank must be analyzed after the CCV. The first CB of a run is labeled as MB.

15.8.1 The acceptance criterion is ≤ the ½RL.

15.8.2 When MB/CB contamination is apparent, samples associated with the MB/CB may require reanalysis.

15.8.3 If the measured sample concentration is >10X the MB/CB contamination, the sample does not need reanalysis. If the sample has a concentration < RL, the sample does not need reanalysis. All other associated samples will require reanalysis.

15.9 Laboratory Fortified Blank or Laboratory Control Standard (LFB or LCS): The 100µg/L standard is used as LCS and analyzed with each batch of 20 samples.

15.9.1 The LCS is from an alternate vendor or source than the CCV with a concentration between low and midlevel calibration standards.

15.9.2 The advisory acceptance criterion is +/- 20 % recovery of the true value. Alternatively control chart limits will be established for each element.

15.9.3 If the recovery is outside of the acceptance goal, one rerun is allowed. If the recovery remains unacceptable, re-digestion of the batch may be necessary or the data will be qualified with a comment regarding LCS recoveries outside of acceptance criteria.

15.10 Sample Duplicate: A sample duplicate is performed every ten samples or only at the request of the client.

15.10.1 The Relative Percent Difference (RPD) for sample/duplicate is ≤ 20%.

15.10.2 A reanalysis is required when the allowable RPD limit is exceeded. If the rerun batch has a RPD > 20%, the sample will be flagged as non-homogeneous.

15.11 Matrix Spike and Matrix Spike Duplicate: A pair of matrix spike and matrix spike duplicate are analyzed with every 20 samples.

15.11.1 The MS/MSD spiking standard is freshly prepared every day and is the same source standard as the LCS with a concentration between low and midlevel calibration standards.

15.11.2 The MS recovery limit is 70-130%.

15.11.3 If matrix spike recovery falls outside the method established limits of 70-130% and lab performance is otherwise in control, then the data may be labeled suspect due to matrix effects.

14.0 References

16.1 USEPA, Methods 6020A, Revision 1, January 1998.

16.2 PerkinElmer Elan ICP-MS DRC II User Manual – Revision 3.

Table 1: Quality Control Requirements

Description	Frequency	Criteria	Corrective Action
Initial Calibration	As needed	$r \geq 0.995$	Re-analyze initial calibration
Calibration Verification (CCV)	Before sample analysis, every 10 injections thereafter, and at end of analytical sequence	%Drift ≤ 10%	Re-analyze CCV. If second re-analysis of CCV fails criteria then perform initial calibration.
CCB	Before sample analysis, every 10 injections thereafter, and at end of analytical sequence	Analyte of interest< ½RL	Re-analyze CCB.
Method Blank (MB)	Ever preparation batch, or 20 samples, whichever is less	Analyte of interest < ½RL	If samples < RL no action. If samples > RL and level in MB is < 5% of the amount found in samples report data with "B" qualifier. If samples > RL but < 5% of amount found in samples re-extract and re-analyze batch.
Laboratory Control Sample (LCS)	Ever preparation batch, or 20 samples, whichever is less	% Rec = 80%-120% (advisory) or limits will be set by control charts.	If samples ND and %Rec > 120% no action. If samples > RL and %Rec > 120% report with "J" qualifier. If samples > RL and % Rec < 80% re-extract and re-analyze batch.
Duplicate	Every preparation batch, or 20 samples, whichever is less.	%RPD ≤ 20%	Qualify data.

Figure 2: Data Review Checklist – Trace Metals by EPA 6020A

Date of Analysis: _____

Review Item	Analyst	Reviewer	Comments
Precalibration Rountine and calibration			
3. Tune RSD≤5%? Mass calibration≤0.1AMU from true value? Resolution<0.9AMU at 10% height? Elan daily performance check passed?			
4. ICS is run at the beginning of run or every 12 Hours?			
5. Initial Calibration $r^2 \geq 0.995$?			
6. Initial Calibration Verification (ICV or QCS) ±10%l?			
7. Initial Calibration Blank ≤ ½ RL?			
8. Continuing Calibration Verification (CCV) within ±10%? CCV every 10 samples?			
9. Continuing Calibration Blank (CCB) after every CCV? CCB ≤ ½RL?			
10. Post digestion is run every 20 samples?			
11. Dilution test is run every 20 samples?			
Batch QC			
1. LCS analyzed daily, or every 20 samples, whichever is greater?			
2. LCS recovery 80%-120%? (advisory)			
3. MS/MSD pair analyzed every 20 samples?			
4. MS/MSD recovery 80%-120%? (advisory)			
5. Sample dup every 20 samples? RPD<20%?			
6. IS intensity >30% of original calibration blank?			
Samples			
3. Results within calibration range?			
4. Data entry spot checked at least 10%?			

Reviewer_____ Date:_____

Table-2: Daily Performance Check

Parameter	Requirement
Mg	>8,000
In	>40,000
U	>30,000
Precision	<3%
Ba^{2+}/Ba	<0.03
CeO/Ce	<0.03
Bg level	<2

Table-3: Example Sample Queue

CCV121806A	CCV *mmddy y*A
MB121806A	MB *mmddy*y A
LFB121806A	LFB *mmddyy* A
Samples 1…10	Samples 1…10
Sample 10 Dup	Dup
Sample 10 MS	MS
CCV121806B	CCV *mmddyy* B
CB121806A	CB0 *mmddyy* A
Samples 11...20	Samples 11...20
Sample 20 Dup	Dup
Sample 20 MS	MS
CCV121806C	CCV *mmddyy* C
CB121806B	CB *mmddyy* B

Note: The first sample rerun is labeled as Sample R, and the second rerun is labeled as Sample RR.

Table-4: Calibration Standard Levels

Name	Standards Size	µg/L
ICAL level 1	50µL 1mg/L calibration stock	1µg/L
ICAL level 2	50µL 10mg/L calibration stock	10 µg/L
ICAL level 3	500µL 10mg/L calibration stock	100 µg/L
ICAL level 4	1500µL 10mg/L calibration stock	300 µg/L

	Signature and Title	**Date**
Prepared by:	Jacqueline Knue Chemist III	
Reviewed by:	Vivianne Sawasaki Microbiologist II	
Reviewed by:	Shera Hickman Quality Systems Manager	
Approved by:	Patryce D. McKinney Chief of Laboratory Services	

Periodic Review:

Signature	**Title**	**Date**

This is a controlled document with the most recent updated revision. This SOP should be reviewed on an annual basis. If the SOP is found adequate, the SOP cover page is signed and dated for documenting the review. If major revisions are needed, a new revision will be released with a new signature page. The above signatures reflect periodic review of the Standard Operating Procedure.

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1.0 Scope and Application

This procedure is applicable to the quantitative determination of sub-µg/L concentrations of a large number of elements in various matrices.

2.0 Summary of Method

2.1 Prior to analysis, samples must be prepared in accordance with the EPA method appropriate for analysis. Soils, sediments, biota, and wastes are prepared using microwave digestion method EPA 3051A or hot block digestion method EPA 3050B. Other preparatory methods may be applicable depending on the matrix and/or project requirements.

2.2 This method measures the ions produced by a radio-frequency inductively coupled plasma (ICP). Analyte species originating in the acidified water sample or sample digest are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer (MS). The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences are assessed and valid corrections applied. Interference correction includes compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

3.0 Health and Safety Warnings

3.1 Before performing this SOP, the pertinent MSDS for each chemical should be read.

3.2 Gloves, eye protection glasses, and lab coat should be worn at all times while performing sample extraction steps or handling samples or extracts

3.3 Concentrated nitric acid presents various hazards. It is moderately toxic and extremely irritating to skin and mucous membranes. Use this reagent in a fume hood whenever possible. If eye or skin contact occurs, flush with large volumes of water. All expired stock and working standards must be disposed of by current ADEC EHL protocol.

3.4 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection for UV emissions.

4.0 Interferences

4.1 Isobaric elemental interferences.

4.1.1 Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal mass-to-charge ratio.

4.1.2 Correction factors for isobaric interferences have been identified by the instrument manufacturer (PerkinElmer) and are incorporated into the ICP/MS data system software.

4.1.3 All masses which might affect data quality must be monitored. Isobaric polyatomic ion interference must be recognized and appropriate corrections made to the data.

4.2 Physical interferences.

5.2 Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies.

4.3 Memory interferences.

5.3 Memory interferences or carry-over can occur when there are large concentration differences between samples or standards which are analyzed sequentially.

5.0 Personnel Qualifications

Analysts independently performing this method must complete an Initial Demonstration of Capability (IDC). Analysts who have not completed an IDC may perform the method only under the direct supervision of the Chemistry Supervisor or other qualified staff.

6.0 Sample Collection, Handling, and Preservation

6.1 For the determination of elements in fish tissues, biologists from International Pacific Halibut Commission (IPHC), Alaska Department of Fish and Game (ADF&G), National Oceanic and Atmospheric Administration (NOAA), commercial and/or native fishermen, or other designees will collect samples from predetermined locations. Fillets are homogenized, as per ADEC EHL SOP-Chemistry-005 Fish Tissue Processing. The homogenized tissue is stored in wide mouth jars in the freezer at ≤ -10°C until the day before analysis. It is then thawed in the refrigerator until weighed out. The unused portion is returned to the freezer.

6.2 For the determination of total recoverable elements in soil or other solid samples, samples are stored at 4ºC. The typical sample size is 50g, but may be smaller.

6.3 Digests, processed as per ADEC EHL SOP-Chemistry-029 Acid Digestion of Biota by EPA 3051A, are stored at room temperature for at least 30 days after analysis, or until such time as it is determined that no further analysis is needed. Once confirmed that no more analysis will be needed, the samples may be disposed.

7.0 Equipment and Supplies

7.1 PerkinElmer Model ELAN DRC II ICP/MS or equivalent.

7.2 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

7.3 Analytical balance with capability to measure to 0.0001g for use in weighing solids, preparing standards, and determining dissolved solids in digests or extracts.

7.4 Calibrated pipettes, 50-1000µL, or equivalent.

7.5 An assortment of high quality disposable pipet tips appropriate for the pipet used.

7.6 Labware.

7.6.1 50mL conical tubes, volume checked at 50mL ± 0.2mL per lot by laboratory (PerkinElmer catalog #B0193234 or equivalent).

7.6.2 15mL conical tubes, volume checked at 10mL ± 0.2mL, 5mL ± 0.2mL, and 2mL ± 0.2mL per lot by laboratory (VWR catalog #21008-216 or equivalent).

7.6.3 Narrow-mouth storage bottles, plastic with screw closure.

7.6.4 Squeeze bottle for 1% nitric acid (HNO_3).

8.0 Reagents and Standards

8.1 Reagent water - All references to reagent grade water in this SOP refer to ASTM Type I water (ASTM D1193) or Milli-Q water.

8.2 Reagent Acids.

Nitric acid, Ultrex II Ultrapure Reagent HNO_3, J.T. Baker, VWR catalog number JT6901-2 or equivalent.

8.3 1% Nitric Acid (HNO_3) solution.

Add 20mL concentrated HNO_3 solution to approximately 1500mL Milli-Q (reagent) water. Mix well and dilute to 2000mL with Milli-Q (reagent) water.

8.4 Standards.

8.4.1 Fish Monitoring.

8.4.1.1 Calibration Standard, AccuStandard catalog #ICP-MS-200.8-Cal-1R or equivalent.

8.4.1.2 Calibration Standard, AccuStandard catalog #ICP-MS-200.8-Cal2-1 or equivalent.

8.4.1.3 6020 Tuning Solution (1ppb ELAN 6100DRC setup solution), Inorganic Ventures catalog #6020TS or equivalent.

8.4.1.4 6020 Internal Standard, Inorganic Ventures catalog # 6020ISS or equivalent.

8.4.1.5 1000µg/mL Magnesium (Mg), Inorganic Ventures catalog #CGMG1-1 or equivalent.

8.4.1.6 1000µg/mL Indium (In), Inorganic Ventures catalog #CGIN1-1 or equivalent.

8.4.1.7 1000µg/mL Uranium (U), Inorganic Ventures catalog #CGU1-1 or equivalent.

8.4.1.8 1000µg/mL Cerium (Ce), Inorganic Ventures catalog #CGCE1-1 or equivalent.

8.4.1.9 1000µg/mL Barium (Ba), Inorganic Ventures catalog #CGBA1-1 or equivalent.

8.4.1.10 Initial Calibration Verification (ICV) Standard, Inorganic Ventures catalog #WW-MSCAL-2 or equivalent.

8.4.1.11 Interference Check Solution (ICS), Inorganic Ventures catalog #6020ICS-0A or equivalent.

8.4.2 Full List.

8.4.2.1 Calibration Stock Standard, AccuStandard catalog #ICP-MS-200.8-Cal-1R or equivalent.

8.4.2.2 1000µg/mL Iron (Fe) Standard, AccuStandard catalog #ICP-MS-27N- 0.1X-1 or equivalent.

8.4.2.3 100µg/mL Tin (Sn) Standard, AccuStandard catalog #ICP-MS-63N- 0.01X-1 or equivalent.

8.4.2.4 100µg/mL Lithium (Li) Standard, AccuStandard catalog #ICP-MS-30N- 0.01X-1 or equivalent.

8.4.2.5 6020 Tuning Solution (1ppb ELAN 6100DRC setup solution), Inorganic Ventures catalog #6020TS or equivalent.

8.4.2.6 6020 Internal Standard, Inorganic Ventures catalog #6020ISS or equivalent.

8.4.2.7 1000µg/mL Magnesium (Mg), Inorganic Ventures catalog #CGMG1-1 or equivalent.

8.4.2.8 1000µg/mL Indium (In), Inorganic Ventures catalog #CGIN1-1 or equivalent.

8.4.2.9 1000µg/mL Uranium (U), Inorganic Ventures catalog #CGU1-1 or equivalent.

8.4.2.101000µg/mL Cerium (Ce), Inorganic Ventures catalog #CGCE1-1 or equivalent.

8.4.2.111000µg/mL Barium (Ba), Inorganic Ventures catalog #CGBA1-1 or equivalent.

8.4.2.12Initial Calibration Verification (ICV1) Standard, Inorganic Ventures catalog #WW-MSCAL-2 or equivalent.

8.4.2.13Initial Calibration Verification (ICV2) Standard, Inorganic Ventures catalog #2008CAL-1 or equivalent.

8.4.2.141000µg/mL Iron (Fe) Standard, Inorganic Ventures catalog # CGFE1-1 or equivalent.

8.4.2.151000µg/mL Tin (Sn) Standard, Inorganic Ventures catalog # CGSN1-1 or equivalent.

8.4.2.161000µg/mL Lithium (Li) Standard, Inorganic Ventures catalog # CGLI1-1 or equivalent.

8.4.2.17Interference Check Solution (ICS), Inorganic Ventures catalog #6020ICS-0A-1 or equivalent.

8.5 Recommended Calibration.

8.5.1 Fish Monitoring.

8.5.1.1 6020 Internal Standard (6020ISS/Ge) Working Solution.

In a 15mL conical tube, dilute 0.1mL (100µL) of 1000ppm Germanium (Ge) to 5mL with 1% HNO_3 for a 20ppm final concentration. Add 5mL of 6020ISS for a final volume of 10mL.

8.5.1.2 200ppb Calibration Standard.

Dilute 1.0mL (1000µL) of Calibration Stock Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 50mL with 1% HNO_3.

8.5.1.3 100ppb Calibration Standard (CCV).

Dilute 0.5mL (500µL) of Calibration Stock Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 50mL with 1% HNO_3.

8.5.1.4 10ppb Calibration Standard.

Dilute 0.05mL (50µL) of Calibration Stock Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 50mL with 1% HNO_3.

8.5.1.5 1µg/mL (ppm) Calibration Intermediate Standard.

Dilute 1.0mL of Calibration Stock Standard to 10mL with 1% HNO_3.

8.5.1.6 0.5ppb Calibration Standard (LLICV/LLCCV).

Dilute 0.025mL (25µL) of 1µg/mL (ppm) Calibration Intermediate Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 50mL with 1% HNO_3.

8.5.1.7 Log the Calibration Standards (8.5.1.1 to 8.5.1.6) into Element under Laboratory Standards.

8.5.2 Full List.

8.5.2.1 6020 Internal Standard (6020ISS/Ge) Working Solution.

In a 15mL conical tube, dilute 0.1mL (100µL) of 1000ppm Ge to 5mL with 1% HNO_3 for a 20ppm final concentration. Add 5mL of 6020ISS for a final volume of 10mL.

8.5.2.2 200ppb Calibration Standard.

Dilute 1.0mL of 10µg/mL (ppm) Calibration Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 10mL with 1% HNO_3.

8.5.2.3 100ppb Calibration Standard (CCV).

Dilute 0.5mL (500µL) of 10µg/mL (ppm) Calibration Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 10mL with 1% HNO_3.

8.5.2.4 10ppb Calibration Standard.

Dilute 0.05mL (50µL) of 10µg/mL (ppm) Calibration Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to10mL with 1% HNO_3.

8.5.2.5 1µg/mL (ppm) Calibration Intermediate Standard.

Dilute 1.0mL of Calibration Stock Standard to 10mL with 1% HNO_3.

8.5.2.6 0.5ppb Calibration Standard (LLICV /LLCCV).

Dilute 0.025mL (25µL) of 1µg/mL (ppm) Intermediate Calibration Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 10mL with 1% HNO_3.

8.6 6020 Tuning Solution.

10ppb 6020 Tuning Solution.

Dilute 0.05mL (50µL) of 6020 Turning Solution to 50mL with 1% HNO_3.

8.7 Daily Performance Check Solution.

8.7.1 Daily Performance Stock Solution (1ppm Mg, In, U, & Ce; 10ppm Ba).

Dilute 0.1mL (100µL) 1000µg/mL Mg, 0.1mL (100µL) 1000µg/L In, 0.1mL (100µL) 1000µg/mL U, 0.1mL (100µL) 1000µg/mL Ce, and 1.0mL of 1000µg/mL Barium to 100mL with 1% HNO_3.

8.7.2 Daily Performance Solution (1ppb Mg, In, U, & Ce; 10ppb Ba).

Dilute 0.1mL (100µL) Daily Performance Stock Solution to 100mL with 1% HNO_3.

8.7 Initial Calibration Verification (ICV).

8.7.1 Fish Monitoring.

Initial Calibration Verification (ICV).

Dilute 0.25mL (250µL) of ICV1 Standard and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 50mL with 1% HNO_3.

8.7.2 Full List.

8.7.2.1 20ppm Sn/Li Working Standard.

Dilute 0.2mL (200µL) of 1000µg/mL Sn and 0.2mL (200µL) of 1000µg/mL Li to 10mL with 1% HNO_3.

8.7.2.2 ICV Solution.

Dilute 0.25mL (250µL) of ICV1, 0.25mL (250µL) of ICV2, 0.25mL (250µL) of 1000µg/mL Fe, 0.25mL (250µL) of 20ppm

Sn/Li Working Standard, and 0.25mL (250µL) of the 6020ISS/Ge Working Solution to 50mL with 1% HNO_3.

8.8 Interference Check Solution (ICS).

8.8.1 ICSA (IFA1).

Dilute 0.1mL (100µL) ICS to 10mL with 1% HNO_3 and mix.

8.8.2 ICSAB (IFB1).

Dilute 0.1mL (100µL) ICS and 0.1mL (100µL) Calibration Standard to 10mL with 1% HNO_3.

9.0 Instrument Setup, Calibration, and Standardization

9.1 Open the ELAN software.

9.2 Verify the argon pressure is at least 80 psi.

9.3 Change the sample and drain tubing if necessary. Click on the Devices icon and adjust the Peristaltic pump speed to "Fast". Verify that the tubing is rolling properly, press "Stop", and lock in the tubing.

9.4 Turn on the recirculator.

9.5 Go to the Instrument icon and click on the Front Panel button to open the ELAN control panel. The System Status bar should indicate "Ready" if all system hardware is operating properly and the plasma can be ignited. If the indicator says "Not Ready" or "Comm. error", refer to the operator manual to determine which system component is causing the fault.

9.6 Click on the plasma Start button to ignite the plasma. The ELAN initiates the ignition process, which requires a little over a minute to complete. The progress of the ignition sequence is shown in the ignition sequence status bar.

9.7 After the plasma ignites, place the sample probe into Milli-Q water and allow the instrument to warm up for approximately 30 minutes. If the plasma does not ignite, refer to the manual to troubleshoot and correct the problem.

9.8 To perform a tuning check of the ICP-MS, select the SmartTune icon. A SmartTune Wizard window will appear. Go to File and open "FishDefault.swz". Highlight "Optimization" on the list inside the SmartTune window. Verify the files on the right-hand side of the window match Figure 1. Create and load a new dataset using the current date (ex: 051711a).

9.9 Aspirate the tuning solution. Click on Mass Calibration and Resolution. Check that the criteria on the right side match Figure 2. Right click Mass Calibration and Resolution and select Quick Optimize.

When the sample is done, check the SmartTune Wizard – Summary and SmartTune – Details reports to verify that the mass calibration and resolution passed (mass values are ±0.1amu of the actual mass values and the resolution is less than 0.9 ± 0.1amu).

9.10 Select the Method icon and load the EPA 6020A 1Daily PerformanceA.mth. Click the Sample icon, Manual tab, and Analyze sample. The Daily Performance Report will automatically print. Confirm that the Net. Intens. RSD of all analytes (Li, Co, In, & Tl) is less than 5%. If this parameter is not met, re-perform the Mass Calibration and Resolution and/or consult the operator manual.

9.11 Aspirate the Daily Performance Check solution. Open the SmartTune Wizard and select Daily Performance Check. The Method File should be "C:\Elandata\Method\Daily Performance.mth". Right-click to Quick Optimize. After the optimization is complete the SmartTune Wizard – Summary and SmartTune – Details report will automatically print. Evaluate the Daily Performance Check to determine if suggested criteria are met. The Daily Performance Report will automatically print. Confirm that the Net. Intens. RSD of Mg, In, and U are less than 3%. If necessary, Quick Optimize the Nebulizer Gas Flow [NEB], Lens Voltage, and Auto Lens Calibration while analyzing the daily performance check solution. Save the results of each optimization under the Optimize icon and Manual Adjust. Reanalyze the Daily Performance Check solution by selecting Daily Performance Check, Quick Optimize under the SmartTune icon. If the parameters of the Daily Performance Check still do not meet suggested criteria, consult the operator manual.

9.12 Load "EPA 6020a fish.mth" for fish tissue analysis or "EPA 6020a Full List.mth" for full list under the Method tab. Select the Report tab on the right side of the screen.

9.12.1 In the Report View Section, check the Send to Printer box and load "EPA6020a quant comprehensiveR1.rop" for fish tissue or "6020a quant comprehensive (full list).rop" for full list in the Report Options Template.

9.12.2 Check the Send to File box in the Report to File box and select "EPA6020a quant comprehensiveR1.rop" for fish tissue or "6020a quant comprehensive (full list).rop" for full list as the Report Options Template. Update the Report Filename to the current date (ex: g:\eh\eh-lab\data capture\icpms_datafiles\ 051711a.rep). Select all boxes (Include Titles, Use Delimiter, and Use Separator) in the Report Format section. Choose Append in the File Write Option box. Select "file" and save method.

9.13 Click the Sample icon and batch tab. Save the sample batch with today's date (ex: 051711a, 051711b, etc). Enter the A/S Location and Sample ID for the samples to be analyzed. To calibrate the system select Run Blank, Stds, and Sample under Measurement Action (*) for the first sample. For additional samples select Run Sample for Measurement Action (*). In the Method (*) column choose either "EPA 6020a fish.mth" for fish tissue analysis or "EPA 6020a Full List.mth" for full list. Select Sample for Sample Type (*). After all changes have been made, save the sample batch.

9.14 Select the green R on the left side of the screen to update the analysis files.

9.14.1 Method.

Load the appropriate method for analysis ("EPA 6020a fish.mth" for fish tissue analysis or "EPA 6020a Full List.mth" for full list).

9.14.2 Dataset.

Create a New dataset using the current date (ex: 051711a). Load the dataset with the current date.

9.14.3 Sample.

The sample name should be sample batch with the current date (ex: 051711a, 051711b, etc).

9.14.4 Report Template.

The report template is "EPA6020a quant comprehensiveR1.rop" for fish tissue or "6020a quant comprehensive (full list).rop" for full list.

9.14.5 Tuning.

Select default.tun for tuning.

9.14.6 Optimization.

Choose default.dac for optimization.

9.14.7 Calibration.

Click on the CalibView icon and open a previous calibration and save it as the current date (ex: 051711a, 051711b, etc).

9.14.8 Polyatomic.

Select elan.ply for polyatomic.

9.15 Place the calibration standard tubes into the positions indicated in EPA 6020A quantitative page.

9.16 Return to the Sample icon and batch tab. Highlight the samples to be analyzed, select Build Run List and print.

9.17 Secure autosampler tubing and lock into place. Select Method icon, click the Sampling tab, press the Probe button, choose Go To Rinse, and place the sample probe in the autosampler.

9.18 Verify that the standards and samples are in the correct location on the autosampler and click Analyze Samples. Save tune file.

9.19 If there is difficulty meeting the linear calibration algorithm with $R \geq 0.998$, the instrument will require recalibration and/or maintenance.

10.0 Sample Preparation

10.1 Prepare samples in accordance with EPA methods 200.8 or EPA 3005 through 3052, depending on the matrix and/or project requirements.

10.2 Method Blank (MB, non-digested water samples only) and Calibration Blank.

Fill 50mL of 1% HNO_3 into a sample tube. Refer to the appropriate EPA method's MB preparation procedure for other matrices.

10.3 Continuing Calibration Verification Standard (CCV).

100ppb Calibration Standard (8.5.2.3).

11.0 Sample Analysis

11.1 Follow instrument warm up and set up procedure described in section 9.0.

11.2 Set up sample queue (similar to the example in Table 1) and analyze sequence.

11.3 Confirm that all quality control requirements stated in Table 2 are met.

11.4 If there is difficulty meeting the QC requirements, the instrument will require recalibration and/or maintenance.

11.5 Shutdown Procedure.

11.5.1 Immerse the sample probe in 1% HNO_3 for 10 minutes; then rinse in water for 5 minutes.

11.5.2 Take the sample probe out of solution, place on autosampler rack, and wait until spray chamber is dry (no more bubbles leave the bottom of the spray chamber).

11.5.3 In the ELAN software, click on "Instrument", and turn off plasma by pressing "Stop" in the Plasma box.

11.5.4 Unclamp and loosen the sample and drain tubing.

11.5.5 Shut off the recirculator.

11.5.6 If running overnight, the Scheduler can be used to rinse and shutdown the instrument. Consult ELAN Online help for scheduling and automating tasks.

12.0 Data Analysis and Calculations

12.1 Under the main Element window, click on "Laboratory", and select **Data Entry/Review**. An "Enter/Edit Data" window will appear. Click on the batch number. Select the **Data Entry** tab, then **Create** to upload the data into the window below. Select the **Data Tool** button.

12.2 Select PE ELAN-REP (*.rep) from the file type drop-down box in the top right hand corner. Double click the file to be uploaded under *G:\EH\Eh-Lab\Data Capture\ICPMS_DataFiles.* The samples will appear in the upper left window. Highlight samples and QC's analyzed, click **Include**, and select **Done**. **Merge** the files.

12.3 Name any unnamed samples as they are in the Element batch under the **Instrument Data** tab. Click the **Refresh** button. Click on **Save**, choose the Element folder *C:\ELMNT\UserFiles*, and select the "gg" spreadsheet. Answer "Yes" to replace the existing data. Click **Done** and close the Data Tool window.

12.4 In the **Enter/Edit Data window**, change the analyst initials from ZZZ to the actual analyst's initials by clicking the Analyst column header, right-clicking, and selecting "Fill cells". Use the drop-down menu to select the analyst's initials.

12.5 Click **Save** in the Enter/Edit Data window to save the merged files. Click done. Under the Data Review tab, select **Query.**

12.6 The test results will now appear. Select the status column, right click and update the status to Analyzed. Lock the page by clicking on the Lock column header and selecting Lock. Click the Printer icon to print the Data Review report.

12.7 Calculations.

12.8.1 Sample Concentration.

$$\text{Concentration (mg/kg)} = \frac{\mu g}{L} \times \frac{mg}{1000\mu g} \times \frac{L}{1000mL} \times \frac{50mL}{\text{Sample Weight (g)}} \times \frac{1000g}{1kg} \times D_f$$

where:

D_f = Dilution factor. If no dilution was made, D = 1.

12.8.2 Percent Drift (%D).

$$\%\ \text{Drift} = \frac{|C_c - C_t|}{C_t} \times 100\%$$

where:

C_c= Calculated concentration

C_t= Theoretical concentration

12.8.3 LCS Percent Recovery.

$$\%\ \text{Recovery} = \frac{C_s}{C_a} \times 100\%$$

where:

C_s = Observed spike concentration

C_a = Spike Level

12.8.4 Duplicate Relative Percent Difference (RPD).

$$\text{RPD} = \frac{|X_1 - X_2|}{(X_1 + X_2)/2} \times 100\%$$

where:

X_1 = Concentration of sample

X_2 = Concentration of duplicate

13.0 Data and Records Management

13.1 Final results are reported in mg/kg.

13.2 Assemble a data package consisting (in order):

13.2.1 Final report from Element;

13.2.2 Data Review Checklist;

13.2.3 Non Conformance Details report(s) (if applicable);

13.2.4 Element Preparation Bench Sheet;

13.2.5 Element analysis sequence;

13.2.6 ELAN DRC II Sample Run List;

13.2.7 Calibration curve; and

13.2.8 ELAN DRC II EPA6020 quantitative comprehensive report for each sample and QC sample. Then,

13.2.9 Place data package in a sea foam green end tab classification folder. Print a label indicating the Element sequence number, Element batch number, and method performed and affix to the end tab of the folder.

13.3 Submit data package to peer or supervisor for second level review. Upon successful review, the second level reviewer updates the Element batch status to reviewed, and files the data package in the central files.

14.0 Quality Control

14.1 Method QC requirements are summarized in Table 2.

14.2 Initial Demonstration of Capability (IDC).

14.2.1 An IDC must be conducted by each new analyst.

14.2.2 Four passing LCS's must be provided to validate an analyst for this method.

14.3 Method Detection Limit (MDL).

14.3.1 An MDL determination must be conducted annually.

14.3.2 An MDL determination should also be conducted when significant changes in instrument occur, or when a new instrument is purchased for the analysis.

14.3.3 If appropriate, the IDC (14.2), MDL, and LLQC (14.5) determinations may be combined.

14.4 Instrument Detection Limits (IDLs).

14.4.1 IDLs should be determined at least every three months or at a project-specific designated frequency.

14.4.2 IDLs in µg/L are determined by calculating the average of the standard deviations of three runs on three non-consecutive days from the analysis of a reagent blank solution with seven consecutive measurements per day.

14.5 Lower Limit of Quantitation Check (LLQC) Sample.

14.5.1 The lower limits of quantitation should be established for all isotope masses utilized for each type of matrix analyzed, each preparation method used, and for each instrument. These limits are considered the lowest reliable laboratory reporting concentrations and should be established from the lower limit of quantitation check sample and then confirmed using either the lowest calibration point or from a low-level check standard.

14.5.2 The LLQC sample should be analyzed after establishing the lower laboratory reporting limits and on an as needed basis to demonstrate the desired

detection capability. Ideally, this check sample and the low-level calibration verification standard will be prepared at the sample concentration with the only difference being the LLQC sample is carried through the entire preparation and analytical procedure. Lower limits of quantitation are verified when all analytes in the LLQC sample are detected within ±30% of their true value. This check should be used to both establish and confirm the lowest quantitation limit.

15.0 References

15.1 USEPA, Methods 6020A, Revision 1, February 2007.

15.2 ELAN Online Help.

15.3 PerkinElmer ELAN Version 3.0 Software Guide, 2003.

15.4 ELAN DRC II Hardware Guide, 2005.

15.5 AS-93 Autosampler for Atomic Spectrometry User's Guide, P/N 0993-5278 or equivalent.

15.6 Element DataSystem, Version 6 or equivalent.

15.7 Element DataSystem Data Tool, Version 3.590 or equivalent.

Table 1
Example Element Sequence

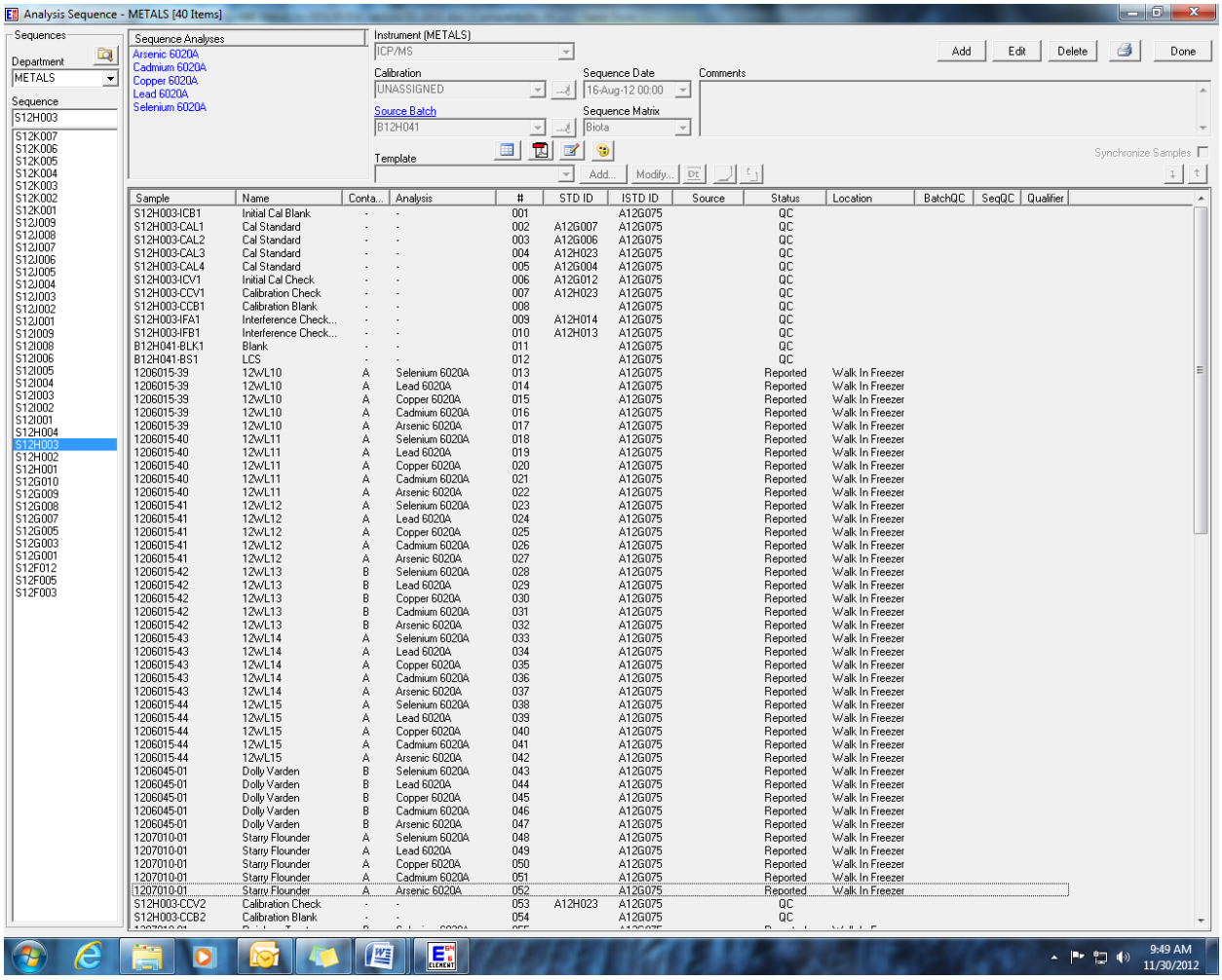


Table 1
Example Element Sequence (cont.)

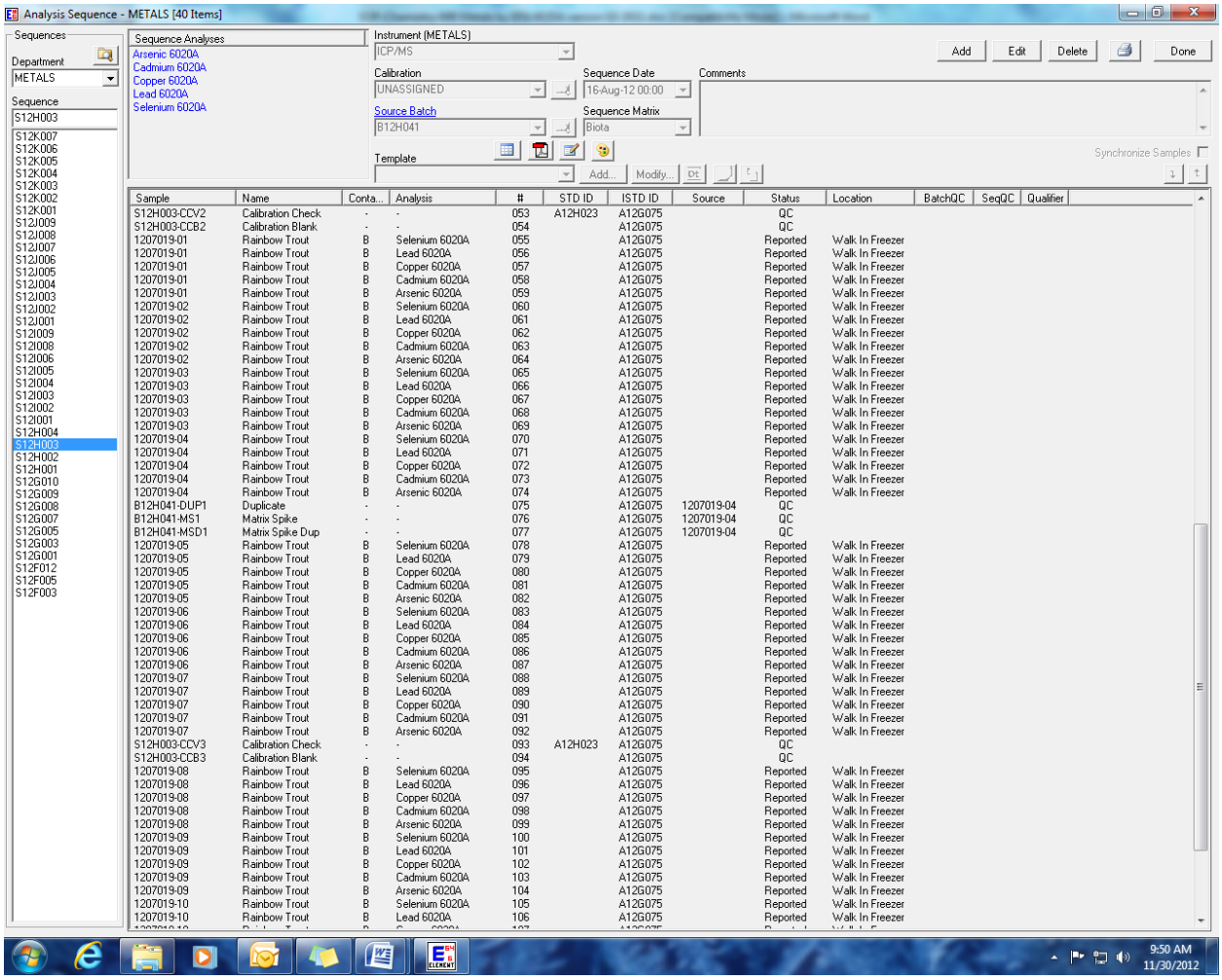


Table 1
Example Element Sequence (cont.)

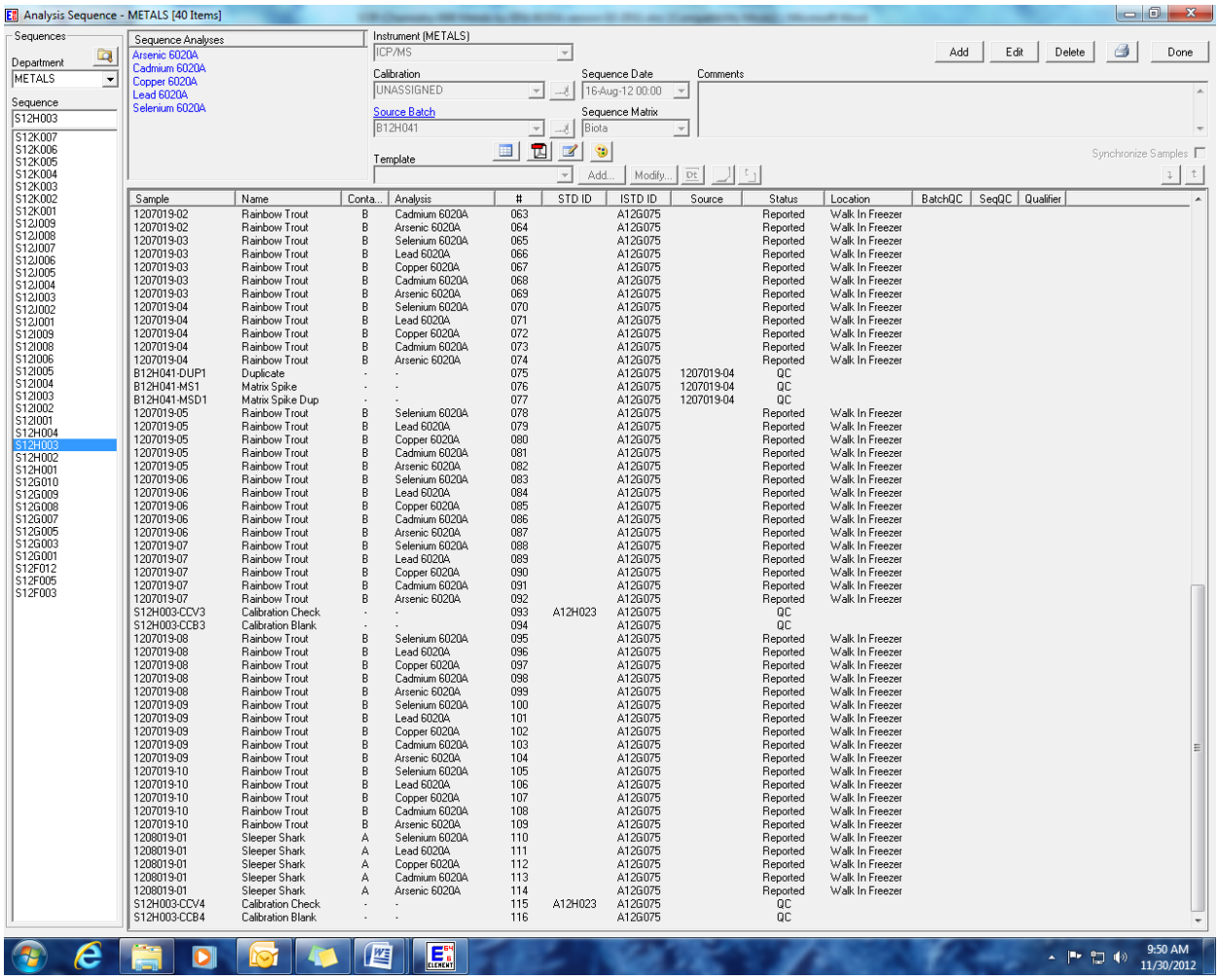


Table 2
Quality Control Requirements

Description	Frequency	Criteria	Corrective Action
Initial Calibration (ICAL)	Daily	$r \geq 0.998$	Re-analyze initial calibration.
Low Level Initial Calibration Verification	Immediately following ICAL	QC % R 70-130%	Recalibrate.

Description	Frequency	Criteria	Corrective Action
(LLICV)			
Initial Calibration Verification (ICV)	Immediately following LLICV	QC % R 90-110%	Recalibrate.
Initial Calibration Blank (ICB)	Immediately following ICV	≤½MRL	Investigate source of contamination and correct the problem. Recalibrate after contamination source eliminated.
Interference Check Sample A (ICSA)	Immediately following ICB	Should be run every 12 hours during active analysis of samples.	Evaluate system for elemental and molecular-ion isobaric interferences. Recalibrate after corrective action completed.
Interference Check Sample B (ICSB)	Immediately following ICSA	Should be run every 12 hours during active analysis of samples.	Evaluate adequacy of inter-element correction factors. Recalibrate after corrective action completed.
Low Level Continuing Calibration Verification (LLCCV)	Every 10 injections **and** at end of analytical sequence	QC % R 70-130%	Re-analyze LLCCV. If second re-analysis of LLCCV fails criteria, then recalibrate. Samples analyzed prior to failing CCV must be reanalyzed for elements that failed criteria.

Table 2
Quality Control Requirements (cont.)

Description	Frequency	Criteria	Corrective Action
Continuing Calibration Verification (CCV)	Every 10 injections **and** at end of analytical sequence	QC % R 90-110%	Re-analyze CCV. If second re-analysis of CCV fails criteria, then recalibrate. Samples analyzed prior to failing CCV must be reanalyzed for elements that failed criteria.
Continuing Calibration Blank (CCB)	Follows every CCV	≤½MRL	Re-analyze CCB. Investigate source of contamination and correct the problem. Recalibrate after contamination source eliminated. Re-analyze samples or qualify results for elements that failed CCB criteria.

Description	Frequency	Criteria	Corrective Action
Method Blank (MB)	Ever preparation batch or 20 samples, whichever is less	≤MRL	If samples < RL, no action. If samples > RL and level in MB is < 5% of the amount found in samples, report data with "B" qualifier. If samples > RL but < 5% of amount found in samples, re-extract and re-analyze batch for elements with blank contamination.
Laboratory Control Sample (LCS)	Ever preparation batch or 20 samples, whichever is less	Limits established by control charts. If no limits established, then use advisory limits %R = 80%-120%.	If samples ND and LCS %Rec > upper control limit, no corrective action. Otherwise, re-digest and re-analyze batch for elements with out of control LCS recoveries.

Table 2
Quality Control Requirements (cont.)

Description	Frequency	Criteria	Corrective Action
Duplicate	Every preparation batch or 20 samples, whichever is less.	%RPD ≤ 20% for analyte values greater than 100x the RL.	Qualify data. Possible non-homogeneous sample.
Matrix Spike/ Matrix Spike Duplicate (MS/MSD)	Every preparation batch or 20 samples, whichever is less	Limits established by control charts. If no limits established, then use advisory limits %R = 75%-125%.	Prepare and analyze post digestion spike addition.
Post Digestion Spike Addition (PS)	Analyze if MS/MSD requirements are unacceptable.	%R = 80%-120%	Dilute sample and re-analyze to compensate for matrix effect.
Dilution (DIL)	Analyze if PS requirements are unacceptable.	Results must agree within ±10% for analyte values greater than 100x the RL	Possible matrix interference. Evaluate need for method of standard additions.
Internal Standard (IS)	Every standard and sample	IS intensity ≥70% of original calibration blank	Dilute sample and re-analyze to compensate for matrix effect.

Table 3
ELAN Smart Tune Wizard

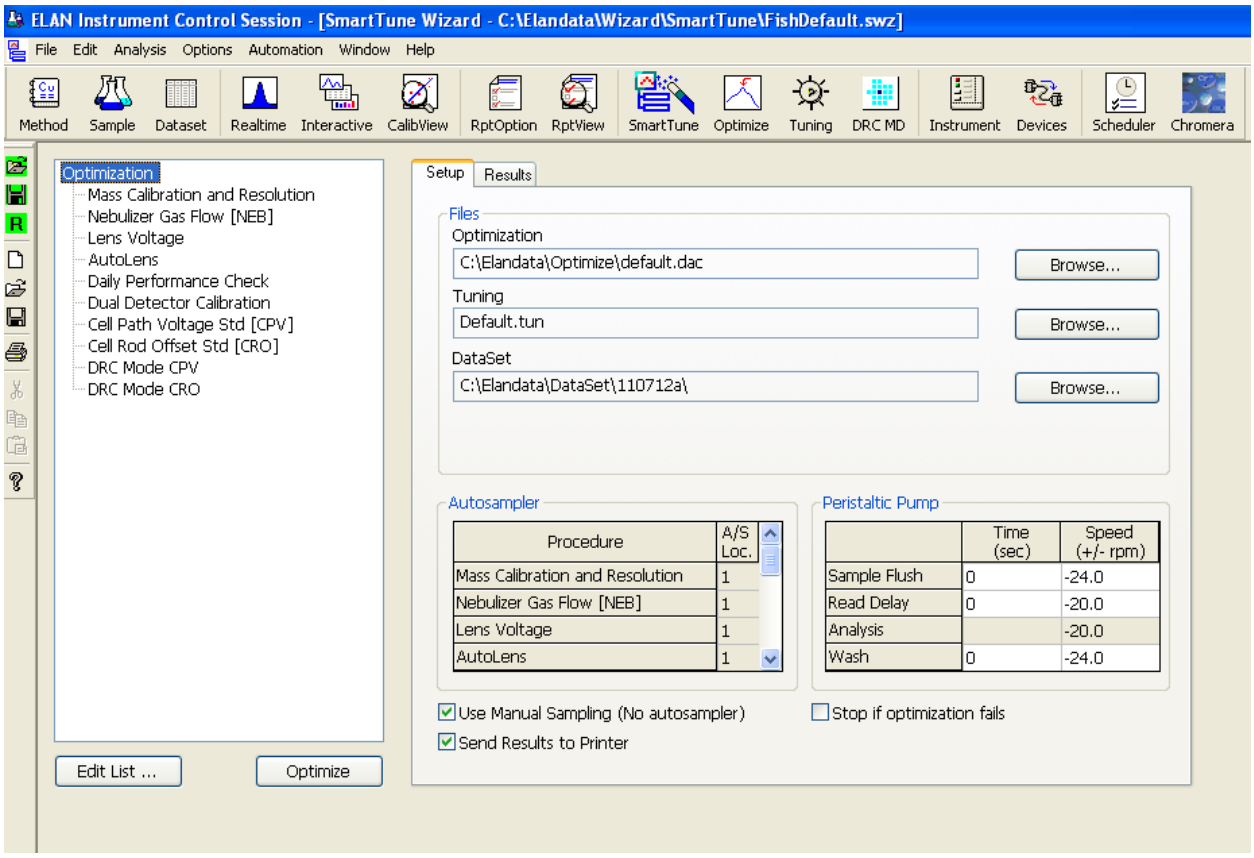


Table 4
Element Data Tool Analyte Cross Table - Fish Monitoring

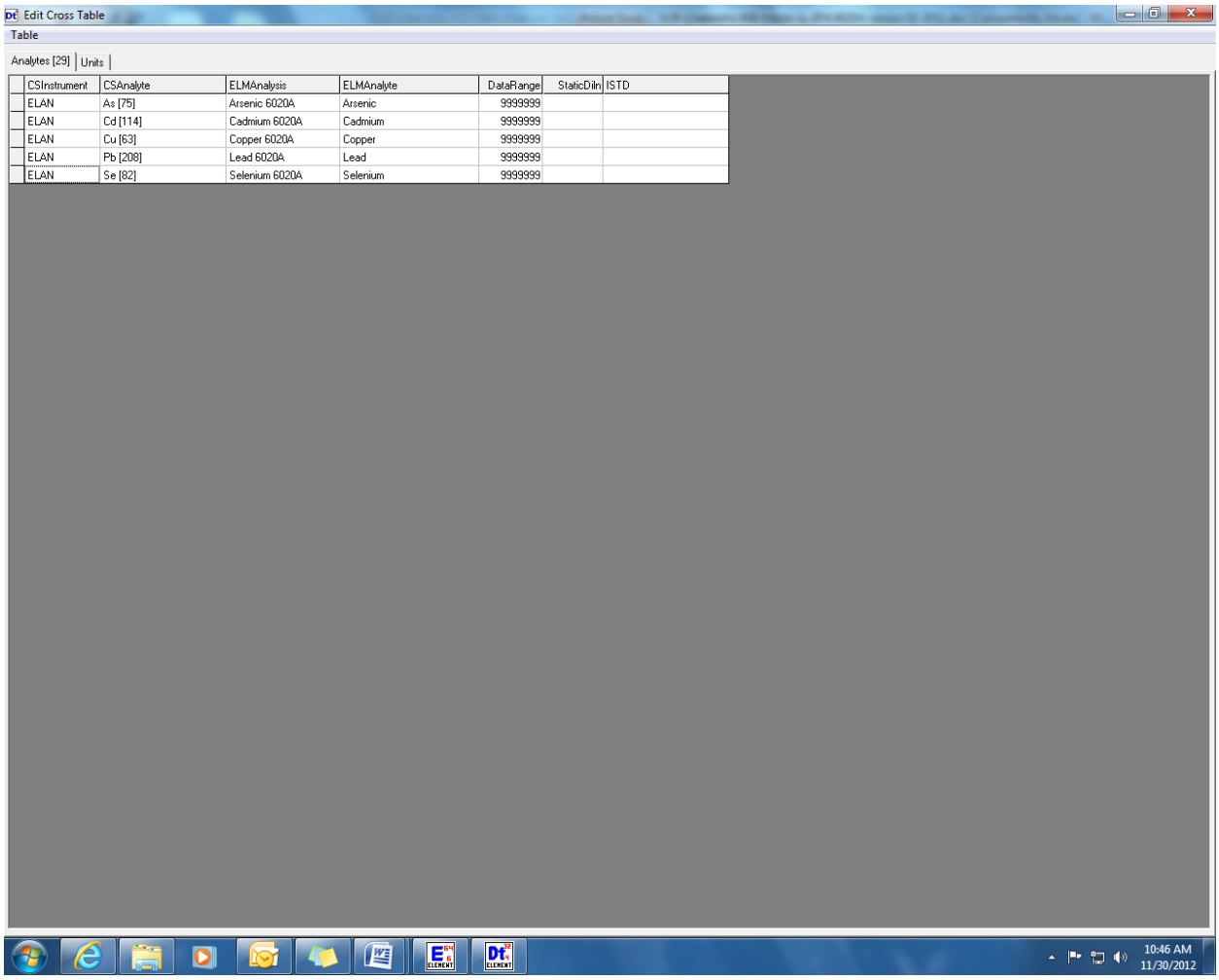
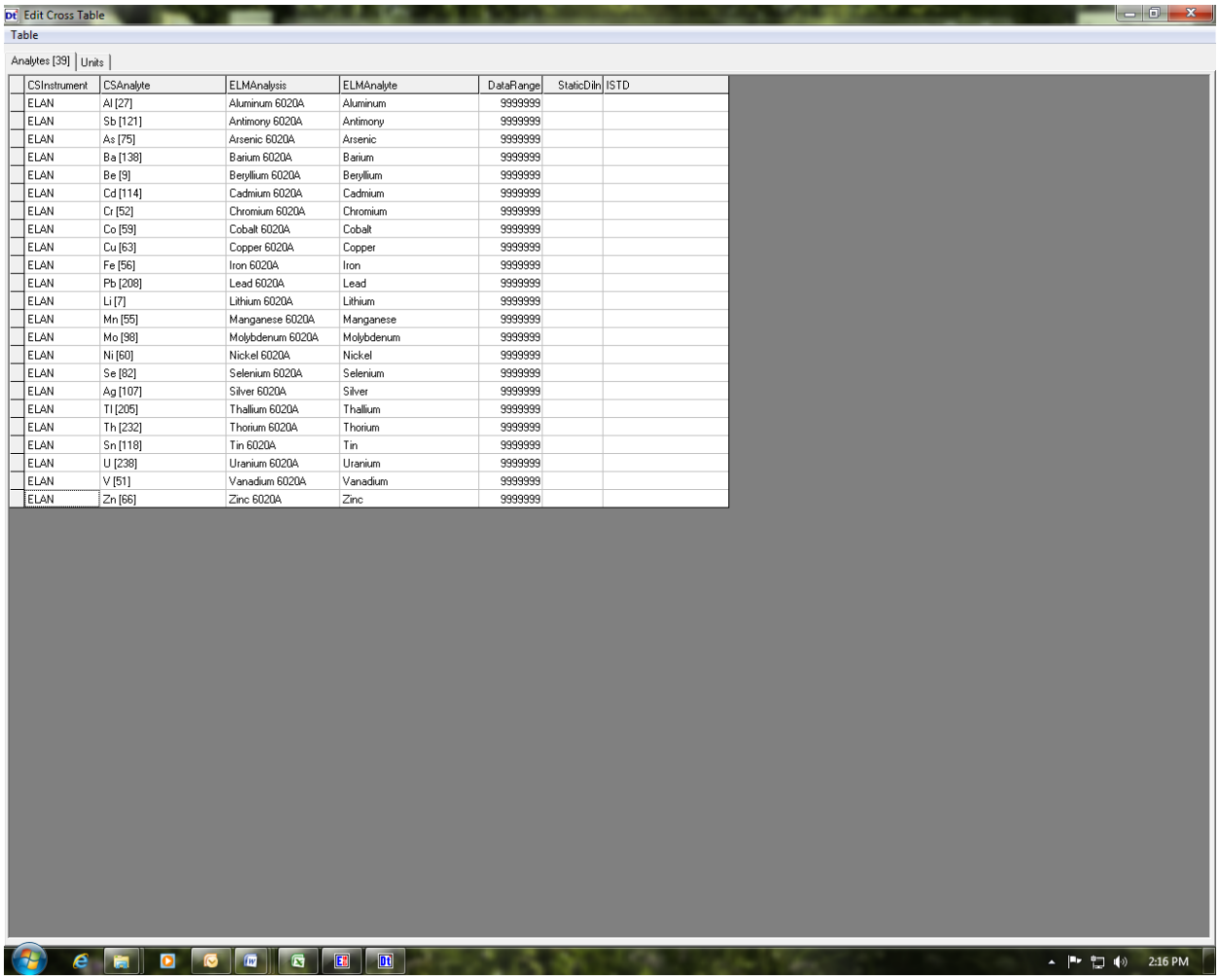


Table 5
Element Data Tool Analyte Cross Table – Full List



CSInstrument	CSAnalyte	ELMAnalysis	ELMAnalyte	DataRange	StaticDiln	ISTD
ELAN	Al [27]	Aluminum 6020A	Aluminum	9999999		
ELAN	Sb [121]	Antimony 6020A	Antimony	9999999		
ELAN	As [75]	Arsenic 6020A	Arsenic	9999999		
ELAN	Ba [138]	Barium 6020A	Barium	9999999		
ELAN	Be [9]	Beryllium 6020A	Beryllium	9999999		
ELAN	Cd [114]	Cadmium 6020A	Cadmium	9999999		
ELAN	Cr [52]	Chromium 6020A	Chromium	9999999		
ELAN	Co [59]	Cobalt 6020A	Cobalt	9999999		
ELAN	Cu [63]	Copper 6020A	Copper	9999999		
ELAN	Fe [56]	Iron 6020A	Iron	9999999		
ELAN	Pb [208]	Lead 6020A	Lead	9999999		
ELAN	Li [7]	Lithium 6020A	Lithium	9999999		
ELAN	Mn [55]	Manganese 6020A	Manganese	9999999		
ELAN	Mo [98]	Molybdenum 6020A	Molybdenum	9999999		
ELAN	Ni [60]	Nickel 6020A	Nickel	9999999		
ELAN	Se [82]	Selenium 6020A	Selenium	9999999		
ELAN	Ag [107]	Silver 6020A	Silver	9999999		
ELAN	Tl [205]	Thallium 6020A	Thallium	9999999		
ELAN	Th [232]	Thorium 6020A	Thorium	9999999		
ELAN	Sn [118]	Tin 6020A	Tin	9999999		
ELAN	U [238]	Uranium 6020A	Uranium	9999999		
ELAN	V [51]	Vanadium 6020A	Vanadium	9999999		
ELAN	Zn [66]	Zinc 6020A	Zinc	9999999		

STANDARD OPERATING PROCEDURE

	Signature and Title	**Date**
Prepared by:	Jodi Estrada Laboratory Technician	
Reviewed by:	Emanuel Hignutt, Jr. Chemist IV	
Reviewed by:	Shera Hickman Quality Systems Manager	
Approved by:	Patryce D. McKinney Chief of Laboratory Services	

Periodic Review:

Signature	**Title**	**Date**

If printed on purple paper or present on the EHL Intranet, this is a controlled document with the most recent updated revision. Uncontrolled copies should be viewed as obsolete and verified upon each use. This SOP should be reviewed on an annual basis. If the SOP is found adequate, the SOP cover page is signed and dated for documenting the review. If major revisions are needed, a new revision will be released with a new signature page. The above signatures reflect periodic review of the Standard Operating Procedure.

Table of Contents

5.3.1 **Page**

18.0 Scope and Application

1.1 This SOP is meant to guide a trained laboratory professional through the procedure of microwave digestion using method EPA 3051A: "Microwave assisted acid digestion of sediments, sludges, soils, and oils" (rev.1 02/07). The digested sample is analyzed by inductively coupled plasma mass spectrometry (PerkinElmer Elan ICP-MS DRC II) for presence of heavy metals. This method is not a total digestion technique for most samples. It is a very strong acid digestion that will dissolve almost all elements that could become "environmentally available." By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment. It is recommended that Milestone's Operator Manual (MA049) for the microwave digester is read and understood before performing any digestions with this equipment.

4.0 Summary of Method

2.1 Ultra-pure reagent grade nitric acid is added to a microwave digestion vessel containing fish tissue. The vessel is then capped, placed in the microwave, and run through a programmed cycle. After cooling, the digested sample is poured into a new, disposable 68mL digestion vessel and brought up to 50mL with Milli-Q water.

5.0 Health and Safety Warnings

3.1 Before performing this SOP, the pertinent MSDS for each chemical should be read.

3.2 Gloves, eye protection glasses and lab coat should be worn at all times while performing sample extraction steps, or when handling samples or extracts.

3.3 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Specifically, concentrated nitric acid presents various hazards and is moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. All expired stock and working standards must be disposed of by current ADEC EHL protocol.

3.4 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

4.0 Cautions

4.1 The analyst must choose an actual sample (not a QC) to be the temperature control (ATC) during the microwave digestion cycle. This sample should be the one the analyst thinks may be the most reactive.

4.2 There must be a minimum of six vessels run during a microwave digestion cycle. If less than six vessels are prepared, additional method blanks should be used as place holders.

5.0 Interferences

5.1 Fish tissue samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed in accordance with the quality control requirements given in this SOP to aid in determining whether method 3051A is applicable to a given sample.

6.0 Personnel Qualifications

6.1 This procedure may only be performed by an EHL Laboratory Technician or Chemist I-V. Analysts independently performing this method must complete an Initial Demonstration of Capability (IDC). Analysts who have not completed an IDC may perform the method only under the direct supervision of the Chemistry Supervisor or other qualified staff.

7.0 Sample Collection, Handling and Preservation

7.1 Fish Tissue: For the determination of elements in fish tissues, biologists from International Pacific Halibut Commission (IPHC), Alaska Department of Fish and Game (ADF&G), Nation Oceanic and Atmospheric Administration (NOAA), and commercial and native fishermen or others will collect samples from predetermined locations. Skinless fillets are homogenized. The homogenized tissue is stored in wide mouth jars in the freezer at $\leq$ -10°C until the day before analysis. Then it is thawed in the refrigerator until it is weighed out. The unused portion is returned to the freezer.

7.2 Digests are stored at room temperature for at least 30 days after analysis or until such time as it is determined that no further analysis is needed. Once confirmed that no more analysis will be needed, the samples may be disposed.

8.0 Equipment and Supplies

8.1 Analytical balance, with capability to measure to 0.0001 g, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

8.2 Air displacement pipettes capable of delivering an assortment of volumes ranging from 50-250µL.

8.3 An assortment of high quality disposable pipet tips appropriate for the pipettes used.

8.4 Milestone Ethos EZ Pro-24 Digestion System with complete rotor assembly.

8.5 Labware

8.5.1 68mL digestion cups and screw caps, volume checked at 50mL ± 0.2mL by vendor (Environmental Express catalog #SC475, or equivalent).

8.5.2 15mL conical tubes, volume checked at 10mL ± 0.2mL and 5mL ± 0.2mL per lot by laboratory (VWR catalog # 21008-216 or equivalent).

8.5.3 Squeeze bottle for 1% Nitric Acid.

8.5.4 Squeeze bottle for Milli-Q (DI) water.

8.5.5 10mL BD Luer-lock tip syringe (VWR catalog #309604, or equivalent).

8.5.6 0.45µm Millex HV syringe filter (Fisher catalog #SLHV033NB, or equivalent).

8.5.7 Disposable polyethylene transfer pipettes (Fisher catalog#13-711-9AM or equivalent).

8.5.8 Plastic spoons.

9.0 Reagents and Standards

9.1 Reagent water - All references to reagent grade water in this SOP refer to ASTM Type I water (ASTM D1193) or Milli-Q water.

9.2 Reagent acid - Nitric acid, Ultrex II Ultrapure Reagent HNO_3 (VWR catalog #JT6901-201, or equivalent).

9.3 1% Nitric Acid (HNO_3) solution.

Add 20mL concentrated HNO_3 solution to approximately 1500mL Milli-Q (reagent) water. Mix well and dilute to 2000mL with Milli-Q (reagent) water.

9.4 LCS Spiking Solution (Inorganic Ventures catalog #WW-MSCAL-2, or equivalent).

9.5 1000ppm Germanium (Inorganic Ventures catalog #CGGE1-1, or equivalent).

9.6 6020 ISS(Inorganic Ventures catalog #6020ISS or equivalent).

10.0 Sample Preparation

10.1 Be sure that all components of the assembly are clean, dry, and intact. Never touch the inner white Teflon vessel with an ungloved hand.

10.2 Remove inner white Teflon vessel from outer brown HTC vessel.

10.3 Rinse the flat end of a plastic spoon with 1% nitric acid and dry with a Kimwipe. Mix the sample thoroughly to achieve homogeneity. All equipment used for homogenization should be cleaned with 1% HNO_3 to minimize the potential of cross-contamination.

10.4 Place the inner vessel on a calibrated balance and weigh out 0.5g ± 0.2g of sample directly into it; record weight to the nearest 0.0001g on the bench sheet print-out. Record vessel number (1-24) next to Element number on bench sheet for tracking purposes. Return inner vessel to outer vessel. DO NOT CAP YET.

10.5 For each batch of 20 samples or less, prepare a Method Blank (MB). Process the Method Blank with the other samples in the batch.

10.6 For each batch of 20 samples or less, prepare a Laboratory Control Sample (LCS), Matrix Spike (MS) and Matrix Spike Duplicate (MSD). Spike the LCS, MS, and MSD with 0.25mL LCS spiking solution and process with the other samples in the batch.

10.7 Add 10mL Ultrex II Ultrapure reagent nitric acid to each inner sample vessel; wait for sample reaction.

10.8 Select one sample (most reactive) to be the **T**emperature **C**ontrol; DO NOT CAP YET.

10.9 Place cap on each inner vessel **except** the **TC (see step below),** hand tighten outer caps on these vessels as well.

10.10 Place assembled vessels on rotor (if haven't already), place cover over rotor/vessel assembly with cover notch facing TC. Microwave door is load bearing and is meant to be a work surface.

10.11 Place specific (ATC) inner cap, probe, TFM foil, and outer cap on TC sample. Slide assembly into microwave, leaving microwave door open.

10.12 Login (Password: 123). Under program tab select “EPA3051.mpr” Press fan icon to make sure probe cable doesn’t catch on anything, press icon again to make it stop.

10.13 Close microwave door and press “Start” button. Be sure microwave door is completely shut. Go to “Run” screen to confirm cycle countdown has begun.

10.14 After cycle is complete, leave samples in microwave for an additional hour to cool down. Carefully move the rotor/vessel assembly onto the door and remove the TC probe, wipe probe with a Kimwipe. Remove cover, place rotor with vessels in hood.

10.15 Point vent on outer vessel towards the back of the hood. Slowly screw the **P**ressure **R**elief **T**ool (PRT) into the top of the outer vessel until all vapors are released. Unscrew the PRT.

10.16 Unscrew the outer vessel cap. Use removal tool to separate the inner vessel from the outer vessel. Remove inner cap, and rinse any residue into the vessel with a squirt bottle of Milli-Q water.

10.17 Transfer sample into labeled 68mL digestion vessel. Rinse inside of inner vessel 3 times with Milli-Q water; swirl, then pour into same 68mL digestion vessel. Bring 68mL digestion vessel volume up to 50mL, cap and lightly shake. Any particulates in the digestate should be removed by filtration, by centrifugation, or by allowing the sample to settle.

10.18 Pour 10mL of the liquid from each tube to a 15mL conical tube. If necessary, adjust the volume using a disposable polyethylene transfer pipette.

10.19 For each batch of 20 samples or less randomly select a sample for preparation of a Post Spike (PS) and Dilution (DIL).

10.19.1 Post Spike (PS)

Add 10mL of the selected sample to a 15mL conical tube and add 0.05mL (50µL) LCS spiking solution. Mix well.

10.19.2 Dilution (DIL)

Add approximately 5mL of 1% nitric acid to a 15mL conical tube and add 2mL of the selected sample. Mix well and dilute to 10mL with 1% nitric acid. Mix well.

10.20 Internal Standard (IS)

10.20.1 20ppm Germanium (Ge) Standard

Add approximately 3mL of 1% nitric acid to a 15mL conical tube and add 0.1mL (100µL) of 1000ppm Ge. Mix well and dilute to 5mL with 1% nitric acid. Mix well.

10.20.2 Internal Standard (IS)

Add 5mL of 6020IS to the 5mL 20ppm Ge Standard for a total volume of 10mL. Spike 0.05mL (50µL) IS into a 10mL sample.

10.21 The samples are now ready for analysis by ICP-MS. Cap and store at room temperature.

10.22 Wash inner vessels in Trace Clean or by alternative microwave method. All other lab ware gets rinsed with Milli-Q water and is dried overnight. Swab the end of the temperature probe holder with a cotton swab.

11 Data Analysis and Calculations

11.1 Refer to the appropriate determinative method for results calculations.

12 Data and Records Management

12.1 All sample batch data are recorded on the Element benchsheet. Record Date/Initials, sample identification, sample weight, final volume, and spiking standards for LCS and MS/MSD samples. Record log numbers for all reagents including acids.

12.2 A copy of Element benchsheet must be included in the completed data package submitted for review.

13 Quality Control

13.1 IDC/MDL Studies: An IDC must be conducted by each new analyst. An MDL study must be conducted annually for the laboratory. If appropriate, the IDC and MDL studies may be combined.

13.1.1 Four passing LCS's must be provided to validate an analyst for this method.

13.1.2 An MDL study should be conducted when significant changes in instrument occur, or when a new instrument is purchased for the analysis.

13.2 Batch QC: A sample batch is defined as a group of 20 or less samples prepared at the same time using a common set of reagents and standards. Each sample batch will include the following batch QC elements:

13.2.1 Method Blank (MB): One MB per batch.

13.2.2 Laboratory Control Sample (LCS): One LCS per batch.

13.2.3 Duplicate (DUP): One Duplicate per batch.

13.2.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD): One MS/MSD pair per batch.

13.2.5 Post Spike: One per batch.

13.2.6 Dilution: One per batch.

13.3 Refer to the determinative method procedure for QC acceptance criteria for each QC type.

14 References

14.1 USEPA, Methods 3050B, Revision 2, December 1996.

14.2 USEPA, Methods 6020A, Revision 1, January 1998.

14.3 USEPA, Method 3051A, Revision 1, February 2007.

14.4 Milestone Pro-16 and Pro-24 Operator Manual (MA049).

14.5 EPA 3050A Acid Digestion SOP (EHL 2011).